

## **Pesticides and Biocides in the Danish Population of European Hedgehogs (*Erinaceus europaeus*)**

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# ABSTRACT BOOK



## SETAC EUROPE 33<sup>RD</sup> ANNUAL MEETING

30 APRIL–4 MAY 2023 | DUBLIN, IRELAND + ONLINE  
“DATA-DRIVEN ENVIRONMENTAL DECISION-MAKING”







# Abstract Book

SETAC Europe 33<sup>rd</sup> Annual Meeting

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This book compiles the abstracts from the 33<sup>rd</sup> annual meeting of the Society of Environmental Toxicology and Chemistry – Europe (SETAC Europe), conducted from 30 April–4 May 2023 in Dublin, Ireland, and online.

The abstracts are reproduced as submitted by the author and accepted by the scientific Committee. They appear in order of abstract code and alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is highlighted in bold.

The information in this abstract book reflects the status of the abstracts as was on 14 April.

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

# About SETAC

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, managers, engineers or others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void and quickly saw dynamic growth in the Society's membership, meeting attendance and publications.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers of the World Council and Geographic Unit Boards of Directors and Councils, and in the composition of committees and other society activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality Through Science®, timely and effective communication of research, and interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Therefore, SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

SETAC's growth is reflected in the founding of Geographic Units around the world. SETAC Europe was established in 1989 as an independent organisation, followed by SETAC Asia-Pacific in 1997 and SETAC Latin America in 1999. In 2002, the four existing organisations joined together under the governance of the SETAC World Council. SETAC Africa is the most recent Geographic Unit, which was adopted in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters and branches have emerged in a number of countries.

SETAC publishes two journals, *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM). ET&C is dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to risk assessment. Integrated Environmental Assessment and Management focuses on the application of science in environmental decision-making, regulation and management, including aspects of policy and law, and the development of scientifically sound approaches to environmental problem solving. Together, these journals provide a forum for professionals in academia, business, government and other segments of society involved in the use, protection and management of the environment for the enhancement of ecological health and human welfare.

SETAC books provide timely in-depth reviews and critical appraisals on scientific subjects relevant to understanding a wide range of contemporary topics pertaining to the environment. These include any aspect of environmental chemistry, toxicology, risk assessment, risk management or environmental policy.

SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1992, and in Brussels, Belgium, established in 1993.

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## Track 1: Environmental and Human Toxicology: From Molecules to Organisms, From Omics to in Vivo

### 1.01.A Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future

#### 1.01.A.T-01 EcoToxChip Test System: A Toxicogenomic New Approach Method (NAM) for Chemical Prioritization and Environmental Management

**Nil Basu<sup>1</sup>**, Markus Hecker<sup>2</sup>, Doug Crump<sup>3</sup>, Jessica Head<sup>4</sup>, Natacha S Hogan<sup>2</sup>, Jianguo Xia<sup>1</sup>, Gordon Hickey<sup>1</sup> and Steven Maguire<sup>5</sup>, (1)McGill University, Canada, (2)Toxicology Centre, University of Saskatchewan, Canada, (3)Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, National Wildlife Research Centre, Ottawa, Canada, (4)Faculty of Agricultural and Environmental Sciences, McGill University, Canada, (5)University of Sydney, Australia

In response to regulatory and stakeholder calls for New Approach Methods (NAMs), since 2016 our team has set forth to develop, test, validate, and commercialize toxicogenomic solutions (i.e., EcoToxChips, and a data evaluation tool, EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. EcoToxChips have been developed for common models (fathead minnow, Japanese quail, *Xenopus laevis*) and native species (rainbow trout, Northern leopard frog, double-crested cormorant). EcoToxChips are now being tested according to a validation plan that has been reviewed by regulatory partners in Environment and Climate Change Canada. To date, more than 1,800 EcoToxChips have been analyzed and in this presentation we will summarize key findings from our case studies. In brief, case studies to date consist of 28 distinct chemicals as well as four complex environmental samples. Two of the chemicals are being tested in a dose-response manner in three independent laboratories. In addition, four of the chemicals are tested in multiple species (species read-across), and four chemicals are being tested both *in vivo* and *in vitro*. For most test chemicals, dose-response (i.e., to derive transcriptomics points of departures) and RNA-seq data (i.e., to evaluate cross-platform comparisons) are available. Exposure studies are complete for 22/28 chemicals and all four complex environmental samples. Notably, 10 of the 28 chemicals are of regulatory interest. The interpretation of EcoToxChip results is facilitated through a user-friendly and intuitive cloud-based tool (EcoToxXplorer.ca) that continues to evolve based on feedback. EcoToxXplorer now integrates with information from the AOPwiki and includes EcoToxModule gene sets designed for interpretation of toxicogenomics data. Taken together, these diverse studies demonstrate how the EcoToxChip Test System can serve as a toxicogenomic New Approach Method that is accessible, standardized, and user-validated. Importantly, many partners have regulatory interests, and so the case studies are helping us to purposefully design the Test System to be one that can be used quantitatively as a decision support tool in regulatory applications including chemical prioritization and guideline development.

#### 1.01.A.T-02 Transcriptomic Profile of Protein-Coding Genes Across Multiple Life Stages of Six Ecologically Relevant Vertebrate Species

**Krittika Mittal<sup>1</sup>**, Jessica Ewald<sup>2</sup>, Doug Crump<sup>3</sup>, Jessica Head<sup>4</sup>, Natacha S Hogan<sup>5</sup>, Markus Hecker<sup>5</sup>, Jianguo Xia<sup>2</sup> and Nil Basu<sup>2</sup>, (1)Natural Resource Sciences, McGill University, Canada, (2)McGill University, Canada, (3)Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, National Wildlife Research Centre, Ottawa, Canada, (4)Faculty of Agricultural and Environmental Sciences, McGill University, Canada, (5)Toxicology Centre, University of Saskatchewan, Canada

As part of the EcoToxChip project, 49 distinct exposure studies were conducted on three lab model species (Japanese quail, fathead minnow, African clawed frog) and three ecologically relevant species (double crested cormorant, rainbow trout, northern leopard frog), at multiple life stages (embryo, adult), exposed to eight chemicals of environmental concern (ethinyl estradiol-EE2, hexabromocyclododecane-HBCD, lead-Pb, selenomethionine-SeMe, 17 $\beta$  trenbolone-TB, chlorpyrifos-CPF, fluoxetine-FLX, and benzo[a]pyrene-BaP). Whole transcriptome analyses were conducted on these samples resulting in a rich RNA-seq dataset covering various species, life stages, and chemicals, which is one of the largest purposeful complications of RNA-seq data within ecotoxicology. Recently, a unified bioinformatics platform of relevance to ecotoxicology, EcoOmicsAnalyst and ExpressAnalyst, was developed to facilitate RNA-seq analysis of nonmodel species lacking a reference transcriptome. The platform uses the Seq2Fun algorithm to map RNA-seq reads from eukaryotic species to an ortholog database comprised of protein sequences from >600 eukaryotic species (EcoOmicsDB) with a translated search. The availability of these tools presents a unique opportunity to examine the EcoToxChip RNA-seq dataset for cross-species comparisons. Accordingly, the objectives were to A) demonstrate the utility of EcoOmicsAnalyst for comparative transcriptomic analysis across multiple datasets, and present a single repository for the EcoToxChip RNAseq dataset, B) conduct integrative analysis of the baseline transcriptomic profile across all species and life stages, and C) look at differential gene expression across all species-life stage-chemical combinations. From the preliminary PCA of the transcriptomic profile, the first principal component captured variability across the tissue types, the second captured variability across the species families, and subsequent principal components captured variability within the species, and effects of chemical exposure. Exposure to TB and HBCD resulted in the fewest number and to EE2 resulted in the greatest number of differentially expressed genes across species. This work shows the potential of the EcoOmicsAnalyst and Seq2Fun platform to facilitate fast and simple analysis of RNA-seq datasets from nonmodel organisms with unannotated genomes and conduct comparative transcriptomic analysis across various species and life stages for cross-species extrapolation.

#### 1.01.A.T-03 Development of a Novel Computational Pipeline to Identify Biomarkers of Reproductive Toxicity in Fish

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Environmental Risk Assessment (ERA) often relies on exposures of live animal to chemicals of concern and the subsequent measurement of adverse outcomes. For example, *in vivo* fish tests are an integral part of chemical regulatory assessment. These assays are necessary to reduce the potential environmental impact of chemicals, but raise ethical concerns, are resource intensive, and offer little mechanistic insight. In the framework of the NC3Rs CRACK IT Challenge “SAFE”, which targets to replace *in vivo* fish studies in environmental risk assessment, we aim to develop *in vitro* assays based on cultured fish cells, focusing particularly on reproductive toxicity.

As a first step toward the development of such *in vitro* assays, which should be robust, sensitive, and scalable, we identified a panel of biomarkers rooted in a holistic understanding of reproductive toxicity in fish. To do this, we set out to develop three complementary methods to identify biomarkers that are based on mechanistic information, have shown robust regulation by environmentally relevant chemicals, and can be measured by established and scalable methods. (1) We built a computable Adverse Outcome Pathway (AOP) network to anchor our biomarker selection to mechanistic information and to move beyond individual signalling pathways to systems level analysis. (2) We integrated data from several bioinformatics databases into a fish-specific protein-protein interaction network to expand our selection into poorly studied territory. (3) We collated published transcriptomic datasets reporting reproductive adverse outcomes in fish to identify the most consistently regulated genes. With this strategy, we identified 382 unique biomarkers. To demonstrate the effectiveness of our strategy, we measured the expression of 36 of these genes in the rainbow trout liver cell line (RTL-W1) exposed to endocrine disruptors. We detected the expression of 30 of those genes and 27 of those showed chemical regulation, i.e., 90% hit rate for genes that were expressed. With these promising preliminary results, we are now poised to measure the concentration-response of all newly identified biomarkers to a panel of chemicals in fish cell lines and create a computational pipeline to extrapolate *in vitro* responses to *in vivo* outcomes.

#### **1.01.A.T-04 Time-Resolved Acute Toxicity Testing With Fish Cells Under Flow Conditions in the RainbowFlow CHIP Biosensor**

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Research into alternatives to animal experiments for aquatic toxicity testing has produced a number of powerful tools, one of which is the use of permanent cell lines. They have been shown to accurately and reproducibly predict acute toxicity to fish. However, most *in vitro* assays using cell lines are carried out under static exposure conditions and assess cell viability only at a single time point, after chemical exposure. Thus, no information is gained on how toxicity develops over time. In the RainbowFlow CHIP project, we use two epithelial cell lines from rainbow trout (*Oncorhynchus mykiss*) for time-resolved acute toxicity investigation under flow conditions. To this end, we use Electric Cell-substrate Impedance Sensing (ECIS); cells are seeded on an electronic chip, and their adherence creates a resistance to an applied electric current. This resistance reflects the health status of the cells. A decrease in resistance is an indicator for loss of cell viability as can be elicited, for example, by exposure to chemicals. This is a fast and noninvasive method for cell viability assessment, which can easily be automated. As a consequence, cell viability can be monitored in real-time during chemical exposure. Flow conditions are achieved in a microfluidic setup, whereby cells are exposed in a channel through which medium is pumped at a defined rate. The fluid flow creates a shear stress, which is a physiological stimulus to epithelial cells neglected in static setups. The use of multichannel ECIS chips enables the testing of several chemical concentrations in parallel. We show the wide applicability of the system on the basis of model compounds with different physicochemical properties and toxic modes of action. Our results indicate that the two cell lines give similar results for the same chemicals, while different chemicals show different toxicity profiles over time. This proof-of-concept study shows the applicability of the biosensor for concentration dependent toxicity testing under flow conditions and highlights the importance of the additional dimension of time. An extension of this setup for the application to on-line effluent monitoring in the field is also currently being implemented.

#### **1.01.P-Tu001 The 'Omics of Fish Epidermal Mucus**

**Denina B.D. Simmons<sup>1</sup>, Urvi Pajankar<sup>1</sup>, Ameila Del Vecchio<sup>2</sup>, Raina Hubley<sup>1</sup>, Gerald Erick Porche Jr<sup>1</sup>, Kylee Ronnenberg<sup>1</sup> and Theresa Warriner<sup>1</sup>,**  
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Fish epidermal mucus is a dynamic biofluid that covers the integument, protecting the fish from potential threats such as injury and pathogens. Epidermal mucus can be sampled from fish in a nonlethal and relatively noninvasive manner, which could be a valuable refinement for studies on aquatic vertebrates in the lab and in the natural environment. We aimed to develop methods to identify and measure proteins and metabolites in fish epidermal mucus, for low-impact monitoring of threatened and/or protected wild fish populations. We used liquid-chromatography high resolution tandem mass spectrometry to identify and measure proteins and metabolites in mucus samples from rainbow trout to determine the most effective sampling and sample preparation methods. We then used the optimal method to measure more proteins and metabolites in mucus samples from other rainbow trout and fathead minnows to better understand mucus 'omics as a tool for ecotoxicological monitoring studies. We measured >5000 proteins in fish mucus and found that there was minimal influence from temporal variation of proteins and metabolites. However, we did find that proteins were sensitive to contaminant exposure and also environmental stress, where >1500 proteins were significantly affected by nickel. Specifically, proteins involved in immune responses, inflammation, and also the lateral line and



sensing were altered. These results suggest that mucus could be a good indicator for stress, immunological, and behavioural responses.

#### 1.01.P-Tu002 Ecotoxicogenomic Profiles of Thyroid Disruption in Zebrafish Embryos

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Active ingredients of pesticides, biocides, but also industrial chemicals can have adverse effects on environmental organisms, which can threaten populations and have far-reaching consequences for the ecosystem. To avoid such effects, European legislation requires chemical manufacturers to provide data for environmental risk assessment of active substances in order to be registered. Current environmental risk assessment of endocrine disruptors targeting thyroid hormone-related physiological processes requires large numbers of amphibians, which is expensive in terms of both resources and animal consumption. Consequently, alternative approaches to screening compounds are needed, and cost-effective, reliable biomarkers must be identified. 'Omics methods are attractive for collecting consistent high-content data and identifying chemical modes of action for prioritizing risk assessment. In the context of identifying screening-compatible biomarkers for thyroid MoAs, we analyzed early molecular signatures induced by the deiodinase inhibitor iopanoic acid and the thyroid peroxidase inhibitor methimazole in zebrafish embryos. Freshly fertilized zebrafish eggs were exposed to a range of concentrations of the two test compounds according to the Fish Embryo Toxicity Test using lethality, hatching rate, and swim bladder size as endpoints. For transcriptome analysis by RNA sequencing followed by differential gene expression analysis with DESeq2, total RNA was extracted after 96 hpf. Both substances induced a statistically significant decrease in swim bladder width, which is consistent with previous literature. The transcriptomic approach aims to identify candidate biomarkers that can identify and differentiate the mode of action of thyroid hormone disruption. Future screening approaches developed based on such data will allow prescriptive hazard assessment of substances and thus ab initio development of more environmentally friendly substances with less negative impact on the aquatic environment.

#### 1.01.P-Tu003 Toward a High-Throughput New Approach Method for Acute Fish Toxicity: Painting the Rainbow (Trout)

Jo Nyffeler<sup>1,2,3</sup>, Felix Harris<sup>4,5</sup>, Steven Lasee<sup>3,5</sup>, Brett R Blackwell<sup>5</sup>, Jonathan Haselman<sup>5</sup>, John Nichols<sup>5</sup>, Daniel L. Villeneuve<sup>5</sup> and Joshua Harrill<sup>5</sup>, (1)Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany, (2)Department of Cell Toxicology, Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany, (3)ORISE participant at US Environmental Protection Agency, (4)ORAU student services contractor at US Environmental Protection Agency, (5)US Environmental Protection Agency

An *in vitro* method (OECD TG 249) using rainbow trout (*Oncorhynchus mykiss*) gill cells (RTgill-W1) in a plate-reader based viability assay (CV-PR) has been developed as a potential alternative to acute fish toxicity tests. We miniaturized this existing New Approach Method (NAM) from 24-well to 384-well format and added two previously established image-based assays: image-based cell viability (CV-IB) and high-throughput phenotypic profiling (HTPP, 'Cell Painting'). We then tested 233 unique chemicals with the aim to compare the results from this NAM to existing *in vitro* and *in vivo* data.

Cells were seeded at 22,500 cells/well and exposed to the test chemicals for 24 h in a minimal medium. Chemicals were tested at eight concentrations (0.05–100  $\mu$ M) in four independent cultures. The three assays resulted in six endpoints. The lowest potency value was considered as the *in vitro* potency.

Existing *in vitro* data from RTgill-W1 cells were collected for 51 chemicals. The fraction of chemical freely available in the culture wells was estimated using three different models (Armitage 2019, Armitage 2021, Fischer 2017). *In vivo* biotransformation was accounted for with the Arnot 2008 model. Existing *in vivo* mortality data for rainbow trout and fathead minnow (*Pimephales promelas*) was gathered from the ECOTOX Knowledgebase (~11,000 records).

Only 26/51 chemicals were active in literature *in vitro* studies and in the present study, with 14 and 24 chemicals having potency estimates within one and two orders of magnitude, respectively. The overall root mean squared error (RMSE) was 1.2 (on a log<sub>10</sub> mg/L scale).

Overall, 78 chemicals had all the required information for modelling and at least three ECOTOX records. Of those, 45 were active in our NAM. The Armitage 2021 model resulted in the best prediction relative to the existing *in vivo* effects data, with a RMSE of 1.4, and 24 and 39 chemicals had potency estimates within one and two orders of magnitude of the *in vivo* value, respectively. By miniaturizing the existing assay, we were able to generate *in vitro* data for >200 chemicals, compared to data for ~100 chemicals obtained previously. Ongoing efforts include the analytical measurement of the free chemical concentration for 12 exemplary chemicals to better understand which *in vitro* disposition model is the most accurate compared to empirical measurements. In the future, we aim to leverage the HTPP data to identify putative modes-of-action.

*This work does not reflect USEPA policy.*

#### 1.01.B Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future

##### 1.01.B.T-01 Grouping Chemicals Into Mode of Action (MoA) Classes - A Semi-Automated (Neuro)Developmental Toxicity Assay Using Zebrafish Embryos

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The major goal of chemical risk assessment is to identify the potential adverse health or environmental effect of chemicals. In this context, animal testing played a central role in predicting the effect of hazardous chemicals on human health. Although it has been widely used to predict adverse effects in humans, animal testing using adult animals has started to raise many ethical and economic concerns. The project PrecisionTox aims at identifying hazards of chemicals using a suite of nonmammalian alternative model organism and in vitro tests, including the zebrafish embryo test. Embryos of the zebrafish (*Danio rerio*) represent a valid alternative to standard animal testing and align with the 3Rs concept. Furthermore, it is an ideal test system to support high throughput chemical screening. Therefore, the main goal of the present study is to apply a previously developed semiautomated high content workflow to assess the adverse effect of 250 chemicals using automated imaging and behaviour tracking for the assessment of developmental (neuro)toxicity. Fertilized zebrafish eggs were exposed in 96-well plates for 96 hours to chemicals covering a wide spectrum of mode of actions.

(Neuro)developmental toxicity was assessed by monitoring mortality, motor behaviour, and morphological changes in a fully automated approach. Preliminary clustering results for six acrylamides and seven imidazoles. Hierarchical clustering and heatmap analysis confirmed specific effect patterns in zebrafish embryos. Acrylamides showed higher lethality and a clear effect on all motor behaviour endpoints with respect to imidazoles. Imidazoles caused cardiotoxicity as a consequence of a general decrease of the heart rate. While imidazoles were decreasing pigmentation, yolk, and the pericardial size, acrylamides were increasing the body curvature and decreasing eye area, body length, and otolith development. Furthermore, it was still possible to observe differences within the group class. Our preliminary results with imidazoles and acrylamides support that a semiautomated (neuro)developmental toxicity assay with zebrafish can highlight distinct effect patterns. Subsequent assessment of a total of 250 chemicals aims at combining the data of various model organisms including OMIC data.

### 1.01.B.T-02 A High-Throughput Analytical Workflow to Assess Specific Toxicokinetic Behaviour of Xenobiotics in Zebrafish Embryos

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Modern toxicology with the integration of 3Rs calls for new developments or adaptations for *in vitro* and *in vivo* models for better evaluating and predicting chemical toxicity. For this purpose, nonsentient organism (NSO) model species, high-throughput generation of toxicological data, and powerful computational modelling approaches are used to understand which chemicals are toxic and why. Currently, the zebrafish embryo (*Danio rerio*) has been actively explored to establish such predictive models and understand the mechanistic effects of chemicals. The precision of models largely depends on the high content and relevance of the collected toxicological data. In this context, zebrafish embryos can be applied in high-throughput studies providing lethal and sublethal effects or behavioural change due to their small size and transparency. Additionally, toxicokinetic information is essential and highly relevant to estimate the specificity of effects caused by chemical substances. To identify compound classes and class-representative compounds that lead to specific toxicokinetic behaviour in the NSO-based model, we exposed zebrafish embryos to 250 compounds and determined their internal concentrations and biotransformation using an LC-MS/MS-based high-throughput analytical workflow. Preliminary results on the internal concentrations of 30 chemicals in the zebrafish embryo were compared to the predicted internal concentration by partition models between water and the embryos. We further determined the internal concentration-based toxicokinetics for compounds significantly deviating from the model and identified transformation products.

### 1.01.B.T-03 Predicting Ecotoxicity Across Taxa Through Machine Learning

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New approach methods, such as machine learning, have promising potential when applied to animal testing for ecotoxicology. Most of the available literature focuses on the classification of chemicals into toxicity classes (e.g., toxic/nontoxic). This is opposed to regression modelling, which predicts actual toxicity values (e.g., LC50/EC50), which is closer to the outcome of ecotoxicological experiments. Comparing the performance of models across available studies to predict toxicity values is difficult because model performance is highly dependent on the data set and the applied train-test-split. Ideally, data sets would cover a broad range of species and chemicals, but in reality, they are usually limited to single or few species and/or a limited chemical space. The applied train-test-splits have to ensure that a chemical and/or a species is only present in either the train or the test split to avoid information leakage. To motivate machine learning experts without a strong biological background to work on ecotoxicological challenges we provide our clean and well-characterized data set as a potential benchmark. The data set is comprised of effect values (EC50/LC50) for acute mortality ( $\leq 96$  h) in three taxonomic groups: fish, crustaceans, and algae. We describe feature curation and data set assembly and give insights into our modelling approach, a greedy forward feature-selection approach based on Gaussian process (GP) regression. The greedy approach proved superior to models using all available features at once, like least absolute shrinkage and selection operator (LASSO) and GP regression. In other words, a selected subset of features led to better performances than using all available features. We compared four molecular representations (three binary fingerprints and the mol2vec embedding). For all representations, the correlation coefficient  $R^2$  from the predictions of the greedy

approach exceeded 0.60 compared to LASSO and GP regression, which reached an  $R^2$  of 0.50 and 0.55-0.59, respectively. Additionally, we compare our model performances to the state of the art by applying the most prevalent models from literature to our data set. To complete the benchmark data set, we propose a number of prediction challenges that could help answer pressing questions from our field. These range from predictions limited to single species that are dominant in our data set, to predictions inside and across taxonomic groups, to predictions on the whole data set.

#### **1.01.B.T-04 Ongoing Status of the Regulatory Acceptance of New Approach Methodologies in the EU**

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Research on Approach Methodologies (NAMs) has created a sizable toolbox of methods available for regulatory use. With the ban of animal experiments for the cosmetics regulation and Article 13 in REACH to test animals as a last resort, the European regulatory landscape has created a strong incentive for the application of NAMs. Additionally, phasing out animal experiments aligns with the opinion of the general public and has almost unanimous support of the European Parliament as demonstrated in the 2021 Motion for Resolution. However, regulatory application of NAMs remains very limited so far, caused by various roadblocks. Changing them requires joint efforts across the sectors of academia, industry, and government. Main roadblocks were analysed and three areas were identified: (1) NAMs for regulatory science need to be fit for purpose, but often NAMs do not fit well in regulatory testing requirements. Cooperation in the developmental phase, with good communication between developers and users will increase the likelihood of NAMs to be accepted for regulatory purposes. There is a need to establish interfaces between the different sectors that enable and foster cooperation. (2) The acceptance of NAMs by regulators highly depends on the validation and robustness of the methods, which represents a time and cost intensive process. Different strategies have been suggested to overcome this bottleneck. Validation oftentimes falls short in publicly funded research projects, which can be overcome by the adoption of funding schemes. Furthermore, cooperation with industry would help overcome the underfunding of validation. A close cooperation between developers and industry can guide selection of the most promising methods for regulatory application for in-house validation studies. (3) In most cases, NAMs do not represent 1:1 replacements of animal tests. Their application in regulatory science will most likely be realized through weight of evidence approaches or Integrated Approaches for Testing and Assessment (IATA). There currently exist various suggestions from ecotoxicology and toxicology on how to integrate these approaches. Here, two things should be kept in mind: (a) the developed approaches need to align to the regulatory context and regulators should be included in their development and (b) the fields of ecotoxicology and toxicology should align their efforts during the development of the approaches and agree on basic structures for decision and language.

#### **1.01.P-Tu004 Development of Freshwater Planaria as a Risk Assessment Tool**

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In ecotoxicology, the adoption of the 3Rs rule—reduce, refine, replace—aiming to limit the use of vertebrate animals in scientific research, has precipitated the development of new models using invertebrate organisms for bioassays. Their relatively high sensitivity to pollutants makes them promising screening tools for predicting toxicity in mammals.

Freshwater planaria, an invertebrate flatworm, have been shown to be a promising alternative model to assess chemicals that may end up in the aquatic environment. They are distributed worldwide, and easy to collect and to maintain in the lab. Their small size allows medium to high-throughput *in vivo* screening of toxicity. Planaria can enable the evaluation of toxicity of chemicals on many levels: 1) they are nontarget organism; 2) almost all species are endemic to their geographical location; 3) they are good indicators of chemical risk on the aquatic ecosystem; 4) their nervous system shares the same neuronal subpopulations and neurotransmitters as the mammalian brain, making them potential screening tools for predicting pollutant toxicity for mammals. As in mammals, their central nervous system controls motor coordination and behaviour.

Planaria have been shown to exhibit specific behavioural patterns in response to neuroactive molecules, active ingredients of drugs or pesticides, whose neuronal targets are known. Many neuroactive molecules have been tested and have induced various stereotypic behaviours in planaria (Buttarelli et al. 2000). A stereotype is a behaviour that is observed when a planarian is exposed to a specific molecule.

However, stereotypes are currently characterised manually. Automating the identification of these stereotypes would allow the rapid screening of the effects of numerous molecules whose mode of action is not yet known. Thus, the aim of this study is to automate the assessment of planarian stereotypes using Deep Learning.

At the end of the labelling process, we obtained images classified into six categories: Wildtype, C-shape, Screw-like, Snake-like, Contracted, and Bridge-like.

Once the model is trained, the stereotype with the highest probability is the final result displayed. As an output we have a simple-to-use web interface which is: the stereotype with the highest probability, a bar chart with the probabilities of the stereotypes associated with the image, a summary table with the name of the images and the predicted stereotypes, downloadable in .csv format.

#### **1.01.P-Tu005 Sleeping with the Fishes: Haloperidol and $\beta$ -Cyclodextrin Effects on Mobility of Planaria**

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The use of animal models in experimental research has helped to uncover many fundamental principles and supported development of applications relevant to biological research, medical sciences, and toxicology among other fields. The historical reliance of the pharmaceutical industry on animal testing has been under increasing scrutiny due to ethical and scientific reasons,

leading to the development of new alternative approaches based on the “3 Rs principles.” This framework encourages the Reduction of the number of animals used, the Refinement of methods employed, and the Replacement of animal use by nonanimal methods when possible, or by substituting one animal model with another from a less-sentient species lower on the phylogenetic scale, including invertebrates. Consequently, we have explored the use of pond worms, *Planaria* species, to replace higher-order animals in toxicology screening.

An important characteristic of *planaria* which has led to their increasing use in neurotoxicology research is the similarity of their central nervous system to that of vertebrates. In fact, despite their simplicity, *planaria* display cephalisation and possess numerous mammalian-like neurotransmitters including glutamate, dopamine, and serotonin. The aims of our current study were to investigate the pharmacological effects of haloperidol (a dopamine 2 receptor antagonist) on *planaria*, and to assess cyclodextrin's (CD) ability to potentiate the drug's delivery through the *planarian* membrane.

Quantitative assessment of haloperidol and CD's effects was achieved using a *planarian* locomotor velocity assay. First introduced by Raffa's research group, this method is a surrogate to techniques employed for the quantification of rodent behaviours.

Following exposure of *planaria* to each tested solution, the organisms showed haloperidol-induced cataleptic effects which have previously been reported in studies using higher-order animals such as mice and rats, thus demonstrating the utility of the *planarian* model. Interestingly, whilst cyclodextrins have been reported to act as membrane disruptors or penetration enhancers in various biological membranes, here the cyclic sugar reduced the efficacy of the haloperidol and the *planaria* remained mobile.

This is probably due to the formation of a complex between the drug and the CD which effectively prevents the drug from exerting its somnoletic effects.

#### **1.01.P-Tu006 Approaches to Reduce Vertebrate Use in Environmental Risk Assessment** *Emma Danby<sup>1</sup>, David Mayfield<sup>2</sup> and Francis Crawley<sup>1</sup>, (1)Labcorp Early Development, United Kingdom, (2)Labcorp*

Environmental Risk Assessments (ERA), whether it be for crop protection products, industrial chemicals, or pharmaceuticals, can lead to large testing programs and potentially high vertebrate usage. Both fish acute and chronic testing is often required as well as bioaccumulation or biomagnification testing being triggered as part of the Persistent, Bioaccumulative and Toxicity (PBT) assessment. Just these studies can use in excess of a thousand fish depending on study design and replication. When endocrine disruption endpoint testing is also required this figure can increase exponentially.

Whilst this testing is essential to ensure environmental protection, Labcorp have been working on strategies to provide the data required to fulfil the needs of an ERA whilst minimizing animal use. Here we discuss some of the strategies available to us and how we can potentially fulfil an ERA using hundreds less fish.

Such strategies include using the threshold or limit test approaches wherever possible to reduce numbers significantly.

Additionally, we are validating in vitro alternative assays for the fish bioaccumulation testing as well as the fish acute test.

Another approach we are taking is to use our regulatory expertise to write waivers resulting in not needing the testing at all and we will give examples of successful waivers written saving time, money, and animals.

#### **1.01.P Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future**

##### **1.01.P-Tu007 Aquatic Risk Assessment of Plant Protection Products in the Future – Still Protective Without Acute Fish Toxicity Testing?**

*Elena Adams, Sarah Hartmann, Daniel Faber, Yvonne Wolf, Markus Ebeling and Eric Bruns, Environmental Safety, Bayer AG - Crop Science Division, Germany*

In July 2022, there were 451 active substances (a.s.) approved for use in plant protection products (PPPs) in the EU. The current risk assessment (RA) of PPPs for the aquatic environment is based on the evaluation of the toxicity to various taxonomic groups including acute and chronic vertebrate studies with fish. Despite the fact that there are alternative, nonvertebrate methods available which are based on fish embryos and cell lines (OECD TG No. 236 and 249), according to the Commission Regulations (EU) No 283/2013 and No 284/2013, there is still the requirement to perform acute fish toxicity studies for the authorization of PPPs. We critically reviewed the current approach to find out if the aquatic Tier 1 RA is still protective even without fish acute testing as it can be usually covered by the RA of other aquatic taxonomic groups. Therefore, we firstly investigated whether acute fish toxicity endpoints are drivers for the aquatic RA of PPPs by calculating and comparing the regulatory acceptable concentration (RAC) for each aquatic taxonomic group. In a second step, we applied RA refinements (geomean approach or reduced assessment factor) and finally, considered the availability of chronic fish studies and whether the endpoint leading to the lowest RAC is not unbound. For this, we examined all currently in the EU approved a.s. with a published EFSA Conclusion except for natural substances and substances without a reliable database. This led to 228 a.s., 77 fungicides, 22 insecticides, 54 herbicides, nine multitarget pesticides, 19 plant growth regulators, and eight others. For 23 a.s., the lowest RAC was found for fish acute toxicity which equals to ~10% of all investigated substances. However, after refinements using geomeans and assessment factor adaptations, only 15 substances were still driven by the acute fish toxicity. From these, 14 substances did either show an unbound acute fish toxicity endpoint, no available chronic endpoints for fish, or an endpoint from a formulation exhibiting a higher toxicity compared to the a.s. endpoint was used in the RA. Only for one out of 228 substances (0.44%, fungicide) the RAC can be considered as driven by the acute fish endpoint. Based on this evaluation it can be stated that there is huge potential to minimize vertebrate testing and that the aquatic RA for PPPs could be performed without acute fish toxicity studies and still assure protectivity for the aquatic environment including fish.

### 1.01.P-Tu008 Replacing Animal-Derived Components in *in Vitro* Test Guidelines OECD 455 and 487

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The next step to advance *in vitro* (eco-)toxicology is the replacement of animal-derived components like fetal bovine serum (FBS) and rat liver homogenates (S9) in effect-based methods. The substitution of these animal-derived products is advantageous as these components can impact bioassay quality due to inherent product variability.

In a proof-of-concept study, the adaptation of the cell-based *in vitro* OECD test guidelines (TG) 487 (micronucleus test for evaluation of genotoxicity) and 455 (detection of estrogen receptor agonists/antagonists) were evaluated for an animal-component-free methodology. The human cell lines A549 (for OECD TG 487) and ER $\alpha$ -CALUX<sup>®</sup> (for OECD TG 455) were investigated for growth in a chemically defined medium (CDM) without FBS and for the implementation of metabolism via the biotechnological metabolism system ewoS9R and induced rat liver S9 for comparison. Both cell lines were successfully cultivated in CDM, but with increased doubling time. The adaptation of the ER $\alpha$ -CALUX<sup>®</sup> cells could be reproduced within two laboratories.

In a next step, both TGs were conducted with ewoS9R and rat S9 in both CDM and FBS-containing medium. For this, benzo[a]pyrene (B[a]P) was used as model compound as it shows increased genotoxicity and endocrine activity after metabolism. The biotechnological ewoS9R showed potential to replace rat liver S9 in OECD TG 487 in FBS-medium with A549 cells. However, a micronucleus assay in CDM could not be conducted as the cells could not be distributed homogeneously, and the formation of cell aggregates made the detection of micronuclei impossible. The OECD TG 455 for endocrine activity assessment could be adapted to CDM and an increase of estrogenic activity of B[a]P could be determined with the addition of ewoS9R or rat S9. However, in the CDM-variant of the OECD 455, the response intensity of the cells and the sensitivity of the assay was reduced.

All in all, promising results were obtained for adaptation of *in vitro* assays to CDM. The application of CDM in OECD relevant TGs has the potential to reduce the use of FBS in cell cultures in the future, thus improving the reproducibility, quality, and informative value of the bioassay results. Furthermore, it can reduce the ethical concerns of using animal components for *in vitro* assays and strengthen those methods as an alternative to animal experiments.

### 1.01.P-Tu009 Novel Approaches to Assessing Interspecies Variation in Sensitivity to Polycyclic Aromatic Hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring chemicals that are ubiquitous in the environment. Polycyclic aromatic hydrocarbons can become alkylated, and the alkyl PAHs are more abundant in certain environmental matrices. Some PAHs can exert toxicity to fishes by activation of the aryl hydrocarbon receptor (AhR). Although there is evidence that alkylation could increase potency of PAHs, these effects are not well characterized. Additionally, most research has focused on a small number of species of fishes, which likely does not capture interspecies variation in sensitivity, as has been demonstrated for other classes of AhR agonists. However, toxicity testing across numerous species is impractical due to limitations in time, cost, and challenges associated with certain species. The goals of this research were to investigate effects of alkylation on potency of PAHs that are agonists of the AhR, and to assess sensitivity of phylogenetically diverse fishes to AhR receptor activation. As a step toward characterizing how alkylation affects potency of PAHs, zebrafish (*Danio rerio*) embryos were exposed to benz[a]anthracene and three alkyl homologues via microinjection to assess lethality. Alkylation had position-dependent effects on potency. The most potent alkyl PAH, 8-methylbenz[a]anthracene, was 5.6-fold more potent than benz[a]anthracene, 7,12-dimethylbenz[a]anthracene was 1.4-fold more potent, and 4-methylbenz[a]anthracene was equally potent. Next, the potency for activation of the zebrafish AhR2 was quantified using a standardized *in vitro* AhR transactivation assay. These assays yielded relative potencies similar to those observed *in vivo*. To assess interspecies differences in sensitivity, the AhR transactivation assay was performed for each of the four PAHs in eight additional phylogenetically diverse species of fishes. Results showed chemical-specific ranges in sensitivity, with White sucker (*Catostomus commersonii*) being the most sensitive and Northern pike (*Esox lucius*) being the least sensitive to each PAH. The range of sensitivity to AhR activation was 73-fold by benz[a]anthracene, 123-fold by 4-methylbenz[a]anthracene, 417-fold by 8-methylbenz[a]anthracene, and 17-fold by 7,12-dimethylbenz[a]anthracene. Due to the abundance of PAHs in the environment and differences in sensitivity across species, developing molecular tools to assess these toxicities more pragmatically could be essential for more objective ecological risk assessment of these chemicals.

### 1.01.P-Tu010 How Can We Achieve Fish Free Fish Testing in Environmental Risk Assessment?

**Daniel Faber<sup>1</sup>**, Yvonne Wolf<sup>1</sup>, Eric Bruns<sup>1</sup>, Tobias Pamminer<sup>2</sup>, Sebastian Schmidt<sup>2</sup>, Thorsten Leicher<sup>2</sup>, Zhenglei Gao<sup>2</sup>, Michael Boeckers<sup>2</sup> and Xin Li<sup>2</sup>, (1)Environmental Safety, Bayer AG - Crop Science Division, Germany, (2)Bayer AG - Crop Science Division, Germany

In general, to register plant protection products (EU regulation 284/2013) and active substances (EU regulation 283/2013) an environmental risk assessment (ERA) is performed on a set of different *in vivo* studies. To assess the effect on aquatic vertebrates (fish) the acute (OECD 203) and chronic toxicity (OECD 210, if DegT90 > 1day) are routinely performed, which results in a

substantial number of fish being used for testing globally each year. In the EU these data requirements are currently strictly enforced even in cases where such tests will likely not provide additional information (e.g., acute fish toxicity is rarely the most sensitive endpoint driving the ERA; see poster abstract of Adams et al.). To reduce unnecessary suffering of fish (3R principle) several nonanimal testing methods (e.g., FET OECD 236, Fish-gill cell lines OECD 249 and QSARs) have been developed over the past years. Each of those NAMs is capable of predicting some adverse effect in fish, yet a 1:1 replacement for all relevant endpoints—especially for chronic fish testing—is very unlikely and not aimed. Therefore, we aim to combine multiple complementary NAMs into a robust workflow within a digital framework to 1) reliably predict all regulatory relevant aspects of fish toxicity testing and 2) minimize potential NAM specific blind spots in the testing strategy. As a first step it is planned that this approach will be used to design tailor-made regulatory studies for fish that meet both the current regulatory data requirements and avoid unnecessary suffering of fish (e.g., limit tests if feasible, defined concentration range in full dose-response study). By combining those different NAMs the regulatory endpoint will be predicted and besides that, the available data will be used to gather a detailed understanding of the toxic dynamics. Ultimately, we strive for a fully NAM based fish free fish testing strategy for the registration of plant protection products. These efforts are in line with the principles of the 3R (refine, reduce, replace) and the ongoing debate to reduce vertebrate testing for regulatory purposes and wherever scientifically justified possible.

#### 1.01.P-Tu011 Comparative Study on the Intrinsic Clearances of Pharmaceuticals in Rainbow Trout (*Oncorhynchus mykiss*) Subcellular S9 Fractions and 3D Primary Hepatocyte Cultures

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Pharmaceuticals are ubiquitous in the aquatic environment. Many of these compounds are relatively lipophilic (high logK<sub>ow</sub>) and predicted to bioaccumulate in fish. Although there are several *in silico* methods available for nonanimal assessment of bioconcentration factors (BCF), these predictions are sometimes contradictory to *in vivo* BCF data (e.g., OECD305), because of their limited capability to account for the impact of hepatic metabolism of pharmaceuticals. In 2018, the OECD launched new guidelines for determination of *in vitro* intrinsic clearances (CL<sub>int</sub>) using cryopreserved rainbow trout (*Oncorhynchus mykiss*) hepatocytes (RTHEP, OECD319A) or liver S9 subcellular fractions (RT-S9, OECD319B), to facilitate extrapolation of *in vivo* CL<sub>int</sub> and its incorporation into *in silico* BCF models.

In this study, we compared the intrinsic clearances of five pharmaceuticals (clozapine, levomepromazine, naproxen, quetiapine, and sertraline) determined in RT-S9 assays (OECD319B) with those determined in 3D cultures of primary RTHEP (custom culture protocol). These pharmaceuticals (all log K<sub>ow</sub>>3) were selected because of their contradictory predicted vs. *in vivo* BCF data for fish (clozapine, sertraline) or their extensive metabolism in human (levomepromazine, naproxen, quetiapine). The hepatocytes were cultured as scaffold-free 3D spheroids (~65 mm, ca. 50 cells/spheroid) on Aggrewell plates (Stemcell Technologies), because this increased the cellular enzyme activities (7-ethoxyresorufin deethylation, EROD) about four-fold compared with suspension cultures (such as OECD319A). In addition to EROD activity, the maturation of the 3D RTHEP cultures was evaluated based on spheroid morphology. The pharmaceuticals' intrinsic clearances were determined on Day 8. In this study, both RT-S9 fractions (pool of five female and two male fish) and cryopreserved RTHEP (pool of six female fish) were of commercial origin (Primacyt Cell Culture Technology GmbH). The intrinsic clearances of pharmaceuticals in RT-S9 and 3D RTHEP incubations were determined by substrate depletion approach over 3–4 h incubation time and, when metabolized, the kinetic constants were derived as instructed in OECD319A/B. The *in vitro* CL<sub>int</sub> values were then extrapolated to *in vivo* estimates and further to S9-BCF and HEP-BCF according to OECD280 guideline. The extrapolated S9-BCF and HEP-BCF were compared with calculated logK<sub>ow</sub> based BCF and, where available, with *in vivo* BCF.

#### 1.01.P-Tu012 Understanding of Phase Distribution of Ionic Liquids in *in vitro* Cell-Based Systems

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The freely dissolved concentration (C<sub>free</sub>) in *in vitro* cell-based system is important as it is not only the dose available for uptake and thus driving effects, but it is also used for quantitative *in vitro-in vivo* extrapolation. Mathematic models have been developed to predict *in vitro* C<sub>free</sub> using partition coefficients between system compartments, but they have not been validated for permanently charged ionic compounds. We aimed to investigate factors influencing the *in vitro* distribution of ionic liquids in cell-based assays to parametrise a mass balance model for prediction of C<sub>free</sub>. The effects of plastic sorption on concentration stability were evaluated in serum-free medium and medium containing 10% fetal bovine serum (FBS) for ionic liquids with increasing length of the alkyl side chain. The liposome-water partition coefficients (K<sub>lip/w</sub>) and serum albumin-water partition coefficients (K<sub>SA/w</sub>) were experimentally quantified by using a solid supported membrane. Three different modelling approaches to predict K<sub>lip/w</sub> were compared: correlation with a HPLC-retention factor (log k'), polyparameter linear free energy relationship (pp-LFER) and COSMOmic. A WCX 96-well microelution solid-phase extraction (SPE) was applied to isolate the freely dissolved ionic liquids from proteins and lipids and thus bound compounds and the quantified C<sub>free</sub> was compared with the predicted C<sub>free</sub> by the mass balance model. The sorption on plastic plates reduced C<sub>free</sub> in the serum-free medium for compounds with an alkyl chain length of ≥12 carbons while medium concentrations remained constant in the medium containing FBS. These results proved that protein containing medium compensates for chemical depletion by a multiwell plate, allowing avoidance of concentration loss to the plastic plate of *in vitro* system. As expected, the lipid and serum affinity were enhanced by increasing the side chain length but the investigated chemicals generally showed stronger affinity to lipid than proteins. All modelling approaches for K<sub>lip/w</sub> prediction showed a good linearity (R<sup>2</sup>=0.95 for COSMOmic, 0.86 for pp-LFER and 0.91 for correlation with log k', respectively), but the accuracy was the best by COSMOmic. The mass balance model performed well in predicting



the concentration bound to protein and lipid phase for chemicals of low to medium hydrophobicity (within a factor of 10), but it underestimated  $C_{\text{free}}$  by up to three orders of magnitude concerning the most hydrophobic chemicals.

#### 1.01.P-Tu013 Use of Japanese Quail EcoToxChips to Derive Transcriptomic Points of Departure for Chlorpyrifos in a Multilab Early-Life Stage Ring Test

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The field of toxicity testing is increasingly moving toward New Approach Methods (NAMs) that are considered to be more cost-effective, ethical, and efficient. Here, we evaluate the combined use of two NAMs: 1) an early-life stage avian embryo test, and 2) transcriptomic dose-response analysis (TDRA) using a targeted qPCR array (EcoToxChip). Repeatability of results was assessed based on a ring test involving three independent laboratories (McGill University, Environment and Climate Change Canada [ECCC] and U.S. Geological Survey Eastern Ecological Science Center [USGS]). Exposures were conducted according to an avian egg injection protocol that we have proposed for standardization. Briefly, fertilized, unincubated Japanese quail (*Coturnix japonica*) eggs were exposed via air cell injection to DMSO (solvent control) or seven concentrations (0.04-40 µg/g egg nominal) of chlorpyrifos (CPF) on embryonic day 0 (ED0) (10 eggs per dose group). Eggs were incubated at 37.5 ± 0.5 °C and 60 ± 5% humidity until mid-incubation (i.e., ED9). Liver samples from five viable embryos were harvested per dose group at ED9 for hepatic gene expression analysis using the 384-gene Japanese Quail EcoToxChip. Standard operating procedures, reagents, chlorpyrifos dosing solutions, and dose-response analysis methods using EcoToxXplorer ([www.ecotoxxplorer.ca](http://www.ecotoxxplorer.ca)) were common between the three laboratories. The strain of quail used and the qPCR instrumentation differed. Preliminary results from two labs point to a large difference in the number of genes for which benchmark doses (BMDs) could be derived (39 vs 70). Despite this difference, transcriptomic points of departure for the first mode (tPODmode) were similar (20.3 µg/g vs 16.8 µg/g) and corresponded to the tPODmode previously calculated from RNASeq data (21.6 µg/g). All tPODs were below our previously published organismal level LOAEL of 41.1 µg/g for chlorpyrifos in Japanese quail embryos. Overall, our results suggest that performing TDRA on a reduced gene set (i.e., 384-gene EcoToxChips) in an alternative avian testing model is a valid approach that can produce repeatable tPODs, which are protective of adverse apical outcomes.

#### 1.01.P-Tu014 Deriving Fish and Daphnia Toxicity QSARs for Anionic Surfactants by Using Experimental and Computational Membrane-Water Partition Coefficients

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Quantitative Structure-Activity Relationships (QSARs) can be used in the environmental risk assessment (ERA) of chemicals to provide a viable alternative to toxicity testing. Anionic surfactants are one of the most widely used surfactant types in the home and personal care industry. The aquatic toxicity of surfactants is generally recognized as being driven by narcosis which is characterised by the adsorption and penetration of the molecule into cell membranes where it can disrupt vital cell mechanisms e.g., energy metabolism and nutrient/oxygen transport across the membrane. Chemical hydrophobicity, usually described with the octanol-water partition coefficient ( $K_{ow}$ ), is a common proxy for predicting the (aquatic) toxicity of chemicals. However, using  $K_{ow}$  is not appropriate for surfactants, not only due to difficulties in its experimental determination but also due to limitations in its applicability for, particularly ionic, surfactants. Biological membranes are ordered and anisotropic tri-dimensional structures, contrary to octanol being homogenous isotropic media. Membrane-water partition coefficient ( $K_{mw}$ ) has been previously demonstrated as a suitable alternative to  $K_{ow}$  that provides a more biologically relevant descriptor.

For some strongly sorbing surfactants or surfactants with very long chains, where water solubility can be a limiting factor, experimental determination of  $K_{mw}$  can be difficult. To overcome this, and in cases where data on shorter pure homologues are available, computational methods can allow for the extrapolation of  $K_{mw}$  to longer chain lengths. Here, we will demonstrate that the measured values of  $K_{mw}$  can be successfully inter- and extrapolated using a computational predictive method for the homologue series of several subgroups of anionic surfactants. For this purpose, we are using a previously developed coarse-grained simulation method, based on molecular dynamics.

This poster will show how both measured data and simulation methods can be integrated for deriving  $K_{mw}$  values and used further in developing QSARs for predicting the acute toxicity of anionic surfactants to aquatic species. Furthermore, it will emphasize the scientific and regulatory potential of using the well-known *in silico* methods with more biologically relevant predictors in avoiding unnecessary fish testing for describing the (eco)toxicity of groups of chemicals with already well-researched mechanisms of toxicity.

#### 1.01.P-Tu015 Effects of the Marine-Derived Anticancer Drug Prodigiosin on Zebrafish: Integration of *in vitro* and *in vivo* Outcomes

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Chemotherapy is the most common treatment against cancer, where anticancer agents (AAs) are designed to kill fast-growing tumour cells by interfering with the cellular reproduction mechanism. However, they act in both healthy and cancerous cells. Prodigiosin (PG) is a microbial marine-derived anticancer drug with significant therapeutic potential against cancer due to its efficacy in treating cancer cell lines. The environmental consequences arising from the presence of PG, especially in the aquatic environment, are still unknown. In this regard, this study aimed at assessing the *in vitro* effects of PG on ZFL cell lines, linking it with the *in vivo* effects observed in zebrafish (*Danio rerio*) as an *in vivo* model. The main goal is to predict the environmental hazard of this new substance and validate the implementation of *in vitro* approaches in the environmental hazard assessment of AAs. For the *in vitro* assessment, cell viability and morphology were evaluated. For the *in vivo* assessment, endpoints measured in zebrafish embryos and larvae were hatching, mortality, developmental malformations, heartbeat, and locomotion. Prodigiosin was cytotoxic to ZFL cells, presenting an IC<sub>50</sub> value of 68.43 mg L<sup>-1</sup> after 72 h of incubation. Cell viability was significantly reduced by 47.5% and 45.2% at 81 and 162 mg L<sup>-1</sup>, respectively after 24 h, while no significant effects were observed at 16 and 32 mg L<sup>-1</sup>. Prodigiosin led to changes in cell morphology, mainly at higher concentrations. Treated cells were fusiform, seeming to modify their attachment to substrate and microtubule organization. Regarding *in vivo* effects, PG induced mortality in *D. rerio* in a dose-dependent manner, with a LC<sub>50-96 h</sub> of 4 mg L<sup>-1</sup>. Heart rate and larvae size were also affected, along with swimming behaviour alterations. Most observed embryo malformations were edema, scoliosis, and failure to absorb the yolk sac, at concentrations above 124 µg L<sup>-1</sup>. Our data revealed that *in vitro* was, as expected, a more sensitive approach to assessing the effects of AAs in our model species. It also showed the specific cellular mode of action of PG such as cell morphology alterations and microtubule organization, with possible consequences for cell division, which can be integrated to understand *in vivo* observed effects, such as developmental malformations in zebrafish.

#### **1.01.P-Tu016 Effects of Chromomycin A2 on Zebrafish and ZFL Cell Lines: Integrating *in vitro* and *in vivo* Approaches**

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Cancer is still one of the most lethal diseases worldwide. Given the variety of forms cancer manifests in humans, the search for new molecules with anticancer activity, the antineoplastic drugs (AAs), is a constant process. In such a context, particular attention has been devoted to natural AAs molecules. Chromomycins comprise a group of antibiotics produced by *Streptomyces* spp., which has antitumour activity. Chromomycin A2 (CA2) was first isolated in 1957 and has been proven efficient in inducing autophagy in melanoma cell lines. Like other AAs, the mode of action of CA2 encompasses DNA intercalation and nonspecific cytotoxicity. This feature places the AAs in the spotlight of emergent environmental contaminants of concern, which require an assessment of the hazard that they may represent for the environment. This study assessed both *in vitro* and *in vivo* toxicity of CA2 to ZFL cell lines and zebrafish embryos, respectively. For the *in vitro* assessment, cell viability, enzyme activity, and cell morphology were evaluated. For the *in vivo* toxicity assessment, endpoints measured in zebrafish embryos and larvae were hatching, mortality, developmental malformations, heartbeat, locomotion, and markers related to oxidative stress and neurotransmission impairment. Results from the *in vitro* exposure showed that the 72-h IC<sub>50</sub> for cell growth inhibition was 0.23 mg L<sup>-1</sup>, while at 6 mg L<sup>-1</sup>, CA2 impaired cell viability by 50% after 24 h. The catalase (CAT) and acetylcholinesterase (AChE) activities were significantly inhibited at all tested concentrations after 24 h of exposure. The activity of glutathione reductase (GR) and Glutathione-S-Transferase (GST) in ZFL cells was not affected by CA2 exposure. CA2 induced alterations in ZFL cells morphology as observed in immunofluorescence preparations. However, the *in vivo* effects were less pronounced, and mortality was not observed at concentrations up to 2 mg L<sup>-1</sup>, nor were any alterations in larvae's locomotion. The heart rate of ZF embryos was decreased at 500 mg L<sup>-1</sup>. GST was at all tested concentrations, GR activity increased at 1 and 2 mg L<sup>-1</sup>, while AChE increased at 2 mg L<sup>-1</sup>, which may signalise an irreversible impairment of both neurotransmission and antioxidant enzymatic activity. *In vivo* CAT activity was not impaired by CA2 exposure. Overall, CA2 was more toxic to ZFL than to zebrafish embryos. These results are compatible with CA2's highly cytotoxic and nonselective nature.

#### **1.01.P-Tu017 'Omics-Based Fingerprinting of Androgen Disruption in Zebrafish Embryos**

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Endocrine disruptors (EDCs), which are capable of disrupting an organisms endocrine system, are undeniably an increasing concern owing to their developmental and reproductive health impact both in human and the environment. Screening for EDC properties, particularly of active ingredients of pesticides, biocides, or complex mixtures of contaminants, involves a diverse range of species and endpoints that are costly in terms of both resources and animal use. To this end, we applied transcriptomics to identify molecular signatures of androgen hormone signalling interference that underpin physiological effects in zebrafish (*Danio rerio*) embryos. In this study, zebrafish embryos were exposed to different sublethal concentrations of the androgenic steroid trenbolone and the antiandrogenic herbicide linuron as model substances to detect enhanced and suppressed androgen hormone signaling pathways in a modified zebrafish embryo toxicity test (zFET). In comparison to the control group, no significant effects were observed for survival or hatching rate. Application of transcriptomics identified genes involved in androgen synthesis, sexual dimorphism, spermiogenesis, and cholesterol synthesis, such as *star*, *cyp7a1*, *cyp17a1*, *dhcr7*, *srd5a2a*, and *hsd11b2*, to be significantly regulated by trenbolone exposure. Both Linuron and Trenbolone significantly targeted

genes involved in the biosynthesis of steroid hormones, essential for sexual development. This study demonstrates that zebrafish embryos are a suitable vertebrate model not only to identify potential hazards associated with exposure to chemicals that directly affect androgen synthesis, but also to investigate potential endocrine disrupting effects of compounds. Evaluation of the molecular biomarkers identified here in zebrafish embryo-based preregulatory bioassays will aid in the identification of ED hazards of compounds, which may significantly reduce animal testing in higher tier studies.

#### **1.01.P-Tu018 First Steps to Expand the Model *Parhyale hawaiiensis* to Evaluate Genetic Damage in Germ Cells**

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Developing new model organisms for tropical estuarine ecosystems is necessary because this area is particularly vulnerable to anthropogenic influence and climate change. Amphipods have good potential as models because they connect benthic food webs to higher trophic levels and are easily cultivated in the laboratory. The amphipod *Parhyale hawaiiensis* has a circumtropical distribution and have been considered an important model for evolutionary development, regeneration, sensory biology, chronobiology, bioprocessing, and more recently ecotoxicology. Protocols to evaluate acute toxicity, genotoxicity, gene expression, regeneration, and to measure internal concentrations of toxicants are already available. We aim to expand the use of *P. hawaiiensis* developing assays to evaluate the adverse effects of environmental contaminants in germ cells. But oogenesis and spermatogenesis have not been yet described for this species. To describe these processes, organisms in amplexus with females in the last part of their reproductive cycle (hatched juveniles in the brood pouch) were separated and observed daily until neonates were found and a new batch of oocytes was observed in the marsupium, marking the start of a new reproductive cycle. Then, males and females were separated and the organisms were observed daily until they completed the reproductive cycle (around 13 days). For females, embryo number and stage were evaluated, and for males, sperm cells were counted. We observed that neonates took between 13 to 14 days to be born at 24±2°C and females had 9.2±4.2 embryos. Sperm count was expressed in number of spermatozooids per male weight. In general, sperm counts seem to increase until the 6<sup>th</sup> day after female fecundation and then the number becomes stable. The maximum observed value was achieved on the 6<sup>th</sup> day, reaching 811±529 sperm cells/male weight (mg). Our next step will be to evaluate DNA damage in germ cells using comet assay after *in vivo* exposure to reference compounds. In the future, we aim to perform experiments exposing not only males, but also females and/or both before copulation to evaluate possible alterations in embryo number, stage development, and abnormalities.

#### **1.01.P-Tu019 Speeding up Eco'n'OMICs – High Throughput Hazard Assessment by Multiplexed Bead-Based Analysis of Molecular Biomarkers**

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The Fraunhofer Attract project Eco'n'OMICs aims at the 'omics-based identification of molecular biomarkers in different ecotoxicological model organisms, which will enable assessment of the hazard potential of substances more rapidly than conventional tests, while providing qualitative information on the compound's mode of action. For this, ecotoxicological experiments with model organisms such as zebrafish embryos are performed with low, sublethal effect concentrations of well-characterized reference substances. Afterwards, total RNA or protein is extracted for differential gene expression analysis using RNA sequencing or LC-MS-based proteomics, in order to identify potential biomarkers. Recent proof-of-concept studies were promising, but results require further validation. Since using RT-qPCR as the standard method for gene biomarker assessment would be extremely labor- and cost-intensive for a higher number of samples and targets, the Eco'n'OMICs project aims at using a bead-based multiplexing method enabling to quantify up to 80 targets for up to 48 samples at once. To increase testing throughput, the exposure experiments are currently transferred from petri dishes to 96-deep-well-plates. The combination of automated pipetting and automated extraction of RNA allows the processing of 96 RNA samples simultaneously. Roughly estimated, this will result in time savings for the individual steps of testing and assessment: Firstly, in comparison to the regular approach it is possible to perform about five times more exposure experiments in the same amount of time. Secondly, the automated extraction of RNA from 96 samples takes only about two hours while the manual extraction takes at least two days. Thirdly, the protocol for bead-based multiplexed gene expression analysis takes about three days, much of which is incubation time. Assuming four 96-well-plates being processed daily using RT-qPCR, this would take 20 days, not including the necessary controls. Taken together, this approach can save a tremendous amount of time, while also avoiding errors and increasing reproducibility thanks to automation.

#### **1.01.P-Tu020 Developmental Effects of Nanoparticles in *Schmidtea mediterranea***

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The rapid growth of nanotechnology, and the use of nanomaterials in personal care products, food, or other applications, leads to increasing amounts of nanoparticles (NPs) in the environment, where they form a potential risk for living organisms. Although the hazardous effects of several of these particles were identified, in-depth knowledge of the underlying modes of action and their relationship to the physicochemical properties of the particles is still lacking in current risk assessment strategies. Especially for developing organisms, large knowledge gaps exist.

In our study, the regenerative capacity of the freshwater planarian *Schmidtea mediterranea* was used as a proxy to study developmental effects. A large amount of pluripotent adult stem cells allows *S. mediterranea* to regenerate all body tissues, including the nervous system. This makes it possible to study developmental and neurotoxicity *in vivo* in line with the 3R principles and REACH regulations. *S. mediterranea* was exposed to polyvinylpyrrolidone-coated and noncoated silver nanoparticles ((PVP)-Ag-NPs) of 20 nm; titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) of 21 nm; and polystyrene micro- and nanoparticles (PS-MNPs) of 49 nm, 215 nm, 1 µm, and 2 µm. The physicochemical properties of the particles were characterized using transmission electron microscopy (TEM) and dynamic light scattering (DLS).

Particle uptake was confirmed for all particles using confocal microscopy and TEM, but their composition and size affected the toxicokinetic profile. All particle types— (PVP)-Ag-NPs, TiO<sub>2</sub>-NPs, and PS-MNPs— impaired (neuro)regeneration. PS-MNPs had a size and concentration-dependent effect on (neuro)regeneration, regardless of their size with the highest concentration, causing the most pronounced effects. PVP-coating strongly influenced the adverse outcomes of the silver NPs, which again emphasizes the role of the physicochemical characteristics in particle-induced toxicity. Further particle-specific effects were increased mucus production after exposure to TiO<sub>2</sub>-NPs which serves as a defence mechanism. In summary, our study shows general and particle-specific effects of (PVP)-Ag-NPs, TiO<sub>2</sub>-NPs, and PS-MNPs, and indicates the importance of including physicochemical properties in hazard and risk characterization. In addition, the stronger effects on developing organisms cannot be overlooked, and investigating different physiological stages is the way forward in future risk assessment strategies.

#### **1.01.P-Tu021 Transcriptomic Points of Departure for Japanese Quail Exposed to Six Pesticides Using the EcoToxChip Test Method**

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Under the banner of New Approach Methods (NAMs) transcriptomics dose-response analysis (TDRA) has emerged as a promising approach for integrating toxicogenomics data into risk assessments. Here we combine an early life stage (alternative to animal) avian embryo test and a targeted qPCR array (EcoToxChip; n = 384 genes of relevance to environmental toxicology) to determine if 1) transcriptomics points of departures (tPOD) can be derived from this test system; and 2) the tPOD values are protective of levels associated with adverse outcomes. Fertilized, unincubated Japanese quail (JQ) eggs were injected with graded concentrations (100, 32, 10, 3.2, 1, 0.32, 0.1 µg/g egg plus DMSO control) of six pesticides: ethoprophos, carbofuran, trichlorfon, permethrin, glufosinate ammonium, and chlorpyrifos. Phenotypic endpoints including mortality, infertility, embryo mass, and deformity (presence of fatty liver) were assessed at embryonic day 9. Gene level BMDs (benchmark dose) were derived for each chemical. While there were relatively few gene-level BMDs (<25 per chemical), they were lower than levels associated with adverse outcomes. Further, for all test chemicals, investigation of gene- and pathway-level results concurred with expected mechanisms of action. Finally, two of the chemicals were repeated and yielded similar results, thus giving confidence to the test method. In conclusion, the EcoToxChip Test Method can yield gene level BMD values that are protective of levels associated with adverse outcomes, and also give insights into mechanisms of action. However, derivation of tPODs may be challenging with the reduced gene set provided by EcoToxChip thus necessitating more work in this area.

#### **1.01.P-Tu022 Fluid Shear Stress Affects the Metabolic and Toxicological Response of the Rainbow Trout Gill Cell Line RTGill-W1**

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The Rainbow trout gill cell-line (RTgill-W1) is of growing importance as a tool in chemical risk and has been accepted by the Organization for Economic Co-operation and Development (OECD 249 Test Guidelines) as a replacement for fish in acute toxicity tests. In these tests cells are exposed under static conditions. In contrast, *in vivo*, water moves over fish gills generating fluid shear stress (FSS) that alters cell physiology and response to toxicants. The current study uses a specialized 3D printed chamber designed to house inserts and allows for the flow (0.2 dynes cm<sup>2</sup>) of water over the cells. This system was used to assess RTGill-W1 cell responses to FSS in the absence and presence of copper over 24 h. In response to FSS RT-gill W1 cells remained attached to the inserts showed no changes in morphology or transepithelial resistance (TER). Fluid shear stress caused increased gene expression of mechanosensitive channel peizo1 and the Cu-transporter atp7a, elevated reactive oxygen species generation, and increased expression of superoxidase dismutase. Cell metabolism was unaffected by Cu (0.163 µM to 2.6 µM Cu) under static conditions but significantly reduced by FSS + Cu above 0.325µM. Differential expression of metallothionein (mt) a and b was observed with increased expression of mta under static conditions and mtb under FSS on exposure to copper. These findings highlight toxicologically relevant mechanosensory responses by RTGill-W1 to flowing water that may influence toxicological responses.

#### **1.01.P-Tu023 Acute Toxicity Study of 3,5-Dichloroaniline Using RTgill-W1, Fish Cell Line**

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Fish cell line assay is an *in vitro* method to predict the acute toxicity of various chemicals which is an attractive alternative assay to the fish acute toxicity test (OECD TG 203). The rainbow trout (*Oncorhynchus mykiss*) cell line assay with RTgill-W1 cells has been used to predict the toxicity *in vitro*. In the current research, we are presenting results of 18 research trials performed at Jai Research Foundation for standardization of assay. In this experiment, confluent monolayers of the RTgill-W1 cell line were exposed in a completely defined, protein-free exposure medium, L-15/ex, at a range of 3.13 to 100 mg/L for 24 h in 24-well tissue

culture plates with positive control substance, 3,5 dichloroaniline (DCA) recommended in OECD TG 249. Cell viability after exposure were analysed by three fluorescent indicator dyes, measured on the same set of cells, and compared to unexposed cells, which served as control. Combining the cell viability measurements and the measured chemical exposure concentrations, effective concentrations impacting 50% of the gill cells (EC50 value) were calculated based on concentration-response modelling. In this experiment, we were able to standardize the assay according to preset quality criteria. The variation of raw fluorescence data of the two cell-free control wells (one carrying L-15/ex and the other carrying L-15/ex with the highest test chemical concentration, 100 mg/L) were found to be below 20%. The cell viability of the solvent control within the range of 10% compared to negative control, which is within the range of natural background variability. The mean nominal EC50 values for the DCA was found to be 36.8–55.3 mg/L, 16.7–103.3 mg/L, and 19.9–69.6 mg/L for AlamarBlue™, CFDA-AM, and Neutral Red, respectively, which is within two-and-a-half standard deviations (SD) from the EC50 values recommended in OECD TG 249 for the test to be valid. This result is within the range given in the OECD TG 249. This study therefore underlines the robustness of the RTgill-W1 cell line assay and its accurate performance.

#### **1.01.P-Tu024 Using Automated Size Measurement Software as a Possible New Approach Methodology to Support Adverse Outcome Pathway**

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Oxidative phosphorylation (OXPHOS) is the main metabolic pathway, taking place in the mitochondria, that produces energy in the form of ATP. Chemicals that act as mitochondria uncouplers can disrupt the OXPHOS process and can lead to adverse outcomes of regulatory concern such as growth inhibition. Chemical regulatory frameworks (e.g., REACH), aim to reduce traditional animal toxicity tests in favor of alternative testing methods, for hazard assessment of chemicals. Under the adverse pathway (AOP) concept, an AOP has been published linking the uncoupling of OXPHOS to growth inhibition, via a reduction of ATP pool and cell proliferation (OECD project #1.92, AOPWiki, AOP #263). This study aims to utilize new approach methodologies to investigate the sublethal effects of mitochondrial uncouplers on the growth as well as developmental inhibition of zebrafish (*Danio rerio*) larvae. Zebrafish embryos were exposed to different concentrations of the known mitochondria uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP) until 96 hours postfertilization. At the end of the exposure period, lateral images of zebrafish larvae were taken. The FishInspector software (version 1.69) will be utilised to automatically annotate features on the acquired pictures of zebrafish larvae. Subsequently information will be extracted on the effects of exposure to CCCP in different structures of zebrafish larvae such as length and size of the yolk sac area, the pericardium, otoliths, and eye as well as otolith to eye distance, among others. The results will be used to expand the current adverse outcome of AOP263, providing information on the effects of mitochondrial uncouplers on multiple structures of zebrafish larvae as well as to construct a quantitative AOP (qAOP) based on AOP263.

#### **1.01.P-Tu025 Risk Assessment for Amphibia in the EU – How Much Additional Animal Testing Is Adequate?**

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Based on publications on effects of agrochemicals on amphibia, in the EFSA opinion paper on amphibia and reptiles recommendations are made how to test aquatic and terrestrial stages. While the body burden concept allows to compare different ways of exposure and to predict toxic effects also across different stages, even within aquatic stages (comparing the sensitivity of tadpoles and fish), fairly inconsistent endpoints are listed in Appendix K of the supporting document Ortiz-Santaliestra et al. 2017. Based on this dataset, the EFSA Panel proposed to apply an additional assessment factor of “at least 100” if acute fish-endpoints were used as a surrogate for acute amphibia endpoints in the risk assessment for aquatic stages. The dataset Appendix K can however be improved, if matching fish endpoints (of the very formulation used in the amphibian test) are used for the evaluation. These acute fish endpoints are available for virtually every formulation as it is a core data requirement. When updating the database accordingly, only very few cases remained with fish less sensitive than amphibia, and only these would justify such a high additional AF (100) to bridge between fish and amphibian LC50-endpoints. If this additional AF were included in any future Guidance Document, this would result in a very conservative risk assessment, and so trigger many additional experiments on amphibia. This is even clearly encouraged by EFSA (to generate more experimental data with amphibia), until there was more evidence available. However, based on the revision of the existing data (by formulation) we conclude that there is already extensive evidence, suggesting that acute risk to amphibians can be reliably predicted, and hence additional acute animal tests are largely dispensable. Also, an additional assessment factor of 100 to bridge between fish and aquatic amphibia stages should be dispensable.

#### **1.01.P-Tu026 Characterization of Hepatic 3D Spheroids Using Multiphoton Fluorescence Microscopy and 'Omics**

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Due to current regulatory legislation mandate for hazard assessment of a large number of chemicals, testing for toxicity and bioconcentration has become more crucial. As a result, the attempts to develop nonanimal and alternative bioassays, with a focus on the use of cells to replace *in vivo* fish toxicity tests, have increased, with focus on fish. The SPHERTOX project is assessing the rainbow trout (RT) hepatic three-dimensional (3D) spheroids model's suitability as an alternative chronic toxicity- and bioconcentration method with enhanced comparability to *in vivo* test systems in fish. The hepatic 3D RT spheroid system is a novel cell model, requiring comprehensive morphological and physiological characterization. We therefore investigated the

uptake, disposition, and toxicity of chemicals in spheroids by using a high-resolution multiphoton fluorescence microscopy (MFM). Multiphoton fluorescence microscopy was used to visualize cellular and subcellular responses during exposure to sublethal concentrations of copper sulphate, 17  $\beta$ -ethinylestradiol, pyrene, 3,4-dichloroaniline, and carbonyl cyanide m-chlorophenyl hydrazone. More specifically, biomarkers for morphology, area, diameter, sphericity, actin synthesis, cytoskeleton, lipid membranes integrity, bile canaliculi structures, DNA/nucleus staining, viability, metabolic activity, and oxidative stress were assessed. Our findings suggest that the 3D spheroids maintain their morphological and physiological integrity, as well as viability for longer than five weeks postisolation, with no signs of hypoxia at the core. Furthermore, transcriptomics and metabolomics are being explored to unravel the spheroids' physiological complexity and potential use in chemical toxicity studies. The morphology and physiological responses of RT hepatic spheroids observed suggest that this model has significant potential as a future toxicity screening tool for different chemicals.

#### 1.01.P-Tu027 Development of a Modular, Cell Line-Based Framework for the Animal-Free Prediction of Chemical Toxicity to Fish

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Environmental risk assessments are performed to protect our natural environment while maximising benefits that chemicals offer to all aspects of life. Traditionally, these assessments are animal-centric, e.g., fish are used and sacrificed in toxicity studies to identify adverse outcomes elicited by chemicals. These procedures raise ethical concerns but, additionally, are too slow and insufficiently informative to keep up with the rapidly growing need to test chemicals for potential harm. Alternatives to these tests are therefore urgently needed. Our vision is to build an alternative fish framework, which combines permanent fish cell cultures and computational models, to allow accurate predictions of chemical impact without the need for animal experimentation. Pursuing this vision, we have previously demonstrated that it is possible to use cell lines from Rainbow trout (*Oncorhynchus mykiss*) to predict the impact of chemicals on fish survival and growth. As part of the CRACK IT Challenge – SAFE: Innovative Safety Assessment of Fish adverse Effects – funded by the NC3Rs (UK), we now propose to expand these concepts to allow predictions on reproductive toxicity. In a first project phase, we have developed a novel multistage computational pipeline that allowed identification of key molecular players and processes in fish reproductive toxicity. We will now exploit the identified key players and processes to develop scalable biomarker response assays in fish cell lines originating from liver, testis, and ovaries. Upon establishing routine culture of these cells, we will explore them for their constitutive biomarker response levels and then monitor regulation of the biomarkers upon exposure to known reproductive toxicants, negative control chemicals, and chemicals for which reproductive toxicity is thus far ambiguous or unexplored. We will complement gene expression analyses by monitoring cellular biomarkers and processes for comprehensive toxicological profiling. We will then incorporate these responses into a computational scheme to translate the cell-based information into an outcome for fish. A comprehensive set of historical *in vivo* data will serve as a benchmark for reproductive toxicity predictions. We will provide wide access to the knowledge gained and services and products developed to ensure maximal benefits to the 3Rs.

#### 1.01.P-Tu028 Extrapolation of *in vitro* Bioactivity Data to Points of Departure (PODs) Using an *in vitro* Mass Balance Model (IV-MBM V2.0)

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*In vitro* bioactivity and toxicity data are increasingly being generated to facilitate chemical assessment activities. One prominent example is the estimation of Administered Equivalent Doses (AEDs). Administered Equivalent Doses are typically calculated as the ratio of an *in vitro* Point of Departure (POD, e.g., EC<sub>x</sub>,  $\mu$ M) and the predicted steady-state blood or plasma concentration (C<sub>ss</sub>,  $\mu$ M) corresponding to an oral dose of 1 mg/kg/d (i.e.,  $AED = EC_x / C_{ss} \cdot 1 \text{ mg/kg/d}$ ). A key underlying assumption inherent to the calculation of AEDs following this approach is that the *in vitro* POD and steady-state blood or plasma concentration are directly comparable exposure metrics (e.g., identical freely dissolved concentrations). This assumption can be problematic for several reasons stemming from the fact that *in vitro* PODs are almost always reported on a nominal concentration basis. Because the composition of assay medium can be very different from the composition of plasma and blood and other losses can occur *in vitro* (e.g., volatilization), there may be substantial errors and uncertainties between *in vitro* PODs and expected C<sub>ss</sub> with respect to exposure. To assess the potential bias in AED calculations that do not explicitly address bioavailability and *in vitro* disposition, we applied an *in vitro* mass balance model (IV-MBM v2.0) to a set of neutral organic chemicals spanning a wide range of partitioning properties. The model was parameterized to represent *in vitro* test systems with different exposure conditions particularly with respect to the volume fraction of fetal bovine serum (FBS) added. An alternative IV POD denoted the “equivalent blood concentration” was calculated using the model and compared to EC<sub>x</sub>. For semivolatile hydrophobic chemicals, the difference between the AEDs calculated using nominal EC<sub>x</sub>s and *in vitro* PODs estimated using IV-MBM v2.0 can approach two orders of magnitude. There can also be large differences for volatile chemicals due to excessive losses from the *in vitro* test system. On the other hand, differences between AEDs calculated using nominal EC<sub>x</sub>s and *in vitro* PODs estimated using IV-MBM v2.0 are relatively small for more hydrophilic chemicals (i.e., less than a factor of two). The model application demonstrates the importance of considering *in vitro* disposition and bioavailability issues when extrapolating *in vitro* bioactivity data to AEDs and then comparing them to traditional *in vivo* PODs and exposure estimates for risk and safety evaluations.



### 1.01.P-Tu029 Evaluation of Fish Keratocyte Explant Culture as Environmental Immunotoxicity Screening Assay

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2,4-Dichlorophenoxyacetic acid (2,4-D) is the active ingredient in many selective herbicides worldwide that can enter aquatic ecosystems through terrestrial runoff and direct application. 2,4-D is commonly used to control invasive aquatic plants; however, 2,4-D exposure has been associated with sublethal impacts on nontarget organisms, such as fish. Limited research on the impacts of 2,4-D exposure on teleost immune function has been conducted, and the development of a high throughput, *in vitro* assay to screen 2,4-D, and other environmental toxicants, for immune impacts would be highly valuable to the field of aquatic toxicology. Keratocyte cells are one component of fish epidermis that are integral for barrier function, compartmentalization of immune cells, and, in some cases, phagocytic activity. Fish keratocyte explant cultures have been demonstrated to serve as a rapid and low-cost cutaneous wound healing model system which allows for *in vitro* study of cell differentiation, replication, and migration. However, this assay's feasibility and utility as an environmental toxicant screening tool is unknown. To validate this assay, 240 scales were nonlethally extracted from 20 adult male fathead minnows (*Pimephales promelas*), incubated in culture medium for 24 hours, and photographed under a microscope at regular intervals. Using Image J, cell sheet area and leading-edge distance were measured—normalized and not normalized to scale area, scale perimeter, and border length—to determine the most consistent measure of cell growth. Cell sheet area at 6 hours was the most variable measurement, while leading-edge distance at 6 and 24 hours was the most consistent. Next, to model herbicide exposure in freshwater fish, scales were placed in culture medium with different concentrations of 2,4-D ranging from 0.04 to 20,480 ppm (15 concentrations; four replicates per concentration; 12 scales per replicate). Ongoing research will compare *in vitro* results to *in vivo* wound healing experiments to test the applicability of this *in vitro* assay to environmentally relevant *in vivo* scenarios. This comparison will evaluate the potential for keratocyte explant cultures to serve as a valid screening tool for toxicological effects on cutaneous wound healing and may be applied to other environmental toxicants and fish species in the future.

### 1.01.P-Tu030 Development of a Nonanimal Integrated Approach to Testing and Assessment for Acute Aquatic Toxicity Hazard Classification and Labelling

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Aquatic toxicity is an ecotoxicological endpoint which provides important information about a chemical's potential to elicit adverse effect(s) on aquatic organisms. Within regulatory toxicology, three trophic levels are typically considered as a proxy of the ecosystem: fish (representing vertebrates/apical consumers), daphnia (representing invertebrates/primary consumers), and algae (representing producers/plants). Acute aquatic toxicity effects are typically studied using one or more OECD guideline standardised tests including the Fish Acute Toxicity Test (OECD 203); the Fish Embryo Acute Toxicity Test (OECD 236) and the Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay (OECD 249). For animal welfare reasons there have been significant efforts in recent years to reduce or eliminate the number of vertebrates, namely fish, used in ecotoxicological regulatory assessment, for example, by applying the 3Rs principle and replacing *in vivo* tests with *in vitro* assays, such as the RTgill-W1 assay, or by developing Integrated Approaches to Testing and Assessment (IATA) for a given chemical. To build upon this body of work, a modular nonanimal IATA has been created, using the following components: *in silico* predictions, RTgill-W1 assay results, physicochemical parameters, and data concerning the mode/mechanism of action (MoA).

Four peer-reviewed publications were used to compile a curated, high-quality dataset of existing *in vivo* and *in vitro* data and good correlation between *in vivo* and *in vitro* data was observed. The *in vivo* data were used to develop a multiclass quantitative structure activity relationship (QSAR) model and the *in vitro* data used to support the development of other relevant modules. The IATA is designed to provide a categorical output suitable for use within the EU Classification, Labelling and Packaging (CLP) and United Nations Globally Harmonized System of Classification and Labelling (UN GHS) frameworks. Case studies and considerations with respect to species relevance and applicability domain will be discussed within.

### 1.01.P-Tu031 Can Healthy Stem Cells Replace Animal Testing in Assays Addressing Molecular Initiating Events *in vitro*?

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The concern of endocrine disruption due to exposure of xenobiotics has triggered the implementation of effect-based substance testing in the field of environmental legislation. These methods (OECD 229, 230, 234, MEOGRT) use fish as a vertebrate model to measure both molecular initiating events (MIE) such as the activation of the oestrogen receptor and the late effects of exposure such as reduced fertility and fitness as proof of the adverse outcome. The benefit of testing strategies requiring both the MIE, e.g., vitellogenin (VTG) and the corresponding adverse outcome pathway (AOP) (e.g., fertility, condition of offspring) for individual test compounds is questionable.

Based on comparative AOP research on laboratory model fish and riverine/marine species that both present longer life spans, more elaborated capacities for tissue regeneration and repeated gonadal recrudescence, we identified a cell type which seems to be crucial for the healthy seasonal development of the gonads and for tissue regeneration. By a targeted process we could isolate nontumour derived self-renewing cell lines from *Cyprinus carpio* brain. A constitutive Histone 2B-GFP fusion-protein expressing transgenic variant of this cell line was used in transplantation experiments, showing the labelled cells behave like gonad

regenerating cells identified *in vivo*. Based on transcriptome data the cells correspond to mesenchymal stem cells of mammals and express the genes of the estrogen pathway. The available H2B GFP transgenic variant is accepted for micronucleus-based genotoxicity assessment as a surrogate for OECD 487 and enables recording of estrogen induced growth promotion. A battery of transgenic variants reporting different modes of action is under development.

Regarding estrogen endocrine disruption a transgenic variant transactivating an estrogen response element controlled NLS-GFP fusion protein is promising. With this construct it is possible to visualize estrogen receptor activation at the single cell level.

The technique enables monitoring the exogenous activation of intrinsic estrogen receptor by means of high throughput live cell imaging. In contrast to cancer cell or yeast-based transactivation assays there is no need to determine the expression of the transgene, e.g., recording catalytic activity of a transactivated enzyme after cell fracturing.

Based on the comprehensive characterisation of the cell lines, we consider them to be perfectly suited to represent MIE and thus contribute to the replacement of animal experiments.

### **1.01.V Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring Approaches and Avenues for the Future**

#### **1.01.V-01 Reducing the Number of Controls in Fish Early Life Stage Toxicity Tests**

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This project is investigating whether it is possible to use only a solvent control and omit a water control in case a solvent is necessary in Fish Early Life Stage toxicity (FELS; OECD TG 210) tests. The use of only one control would reduce the number of fish used in tests when solvents are required.

Extensive statistical computer simulations have been conducted to investigate the statistical power of the FELS study to determine NOEC and EC<sub>x</sub> values based on one control or pooled controls. The computer simulations covered the observed range of variability in each endpoint observed in each species of fish, the level of variance heterogeneity or overdispersion, the effect of mortality on sublethal responses, and monotonic and nonmonotonic concentration-responses including flat and shallow trends as well as hormesis and quantified the effects of these data characteristics. The study demonstrated the benefits of model averaging on EC<sub>x</sub> estimation over model selection but also explored model selection criteria both for its own uses and to improve model averaging.

Furthermore, control and concentration-response data from FELS tests have been collected for analysis of the effect of the control choice on NOEC and EC<sub>x</sub> values. Simulated and collected test data were used to determine whether, for one or more required endpoints, there are systematic differences between the two controls. For each endpoint, the distributions of control data (means, between-, and within-replicate variances) for water, solvent, and pooled controls were investigated. Fish Early Life Stage concentration-response data were used to investigate the treatment effect (NOEC, EC<sub>x</sub> regressions) when using the water, solvent, or pooled controls.

The analyses indicate that, when the solvent dimethylformamide is used, Fathead Minnow (*Pimephales promelas*) EC<sub>10</sub> point estimates for length and weight based on the solvent only are more accurate (i.e., in agreement with the real EC<sub>10</sub>) than those based on either the water control only or the pooled control and that there was a greater ability to fit models for EC<sub>x</sub> estimation with the solvent control versus the water control. Use of the solvent control maintains 80% power to detect 10% effects with lower false positive rates than other control choices. Results for survival, abnormalities, and time to hatch or swim-up will also be reported.

#### **1.01.V-02 Zebrafish Cell Lines to Assess the Cytotoxic Effect of Single and Joint Exposures of Antineoplastic Agents**

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Over the years, the global average age of the human population has increased. The ageing process is a high-risk factor for the most frequent diseases (e.g., cardiovascular diseases and cancer), leading to an increase in pharmaceuticals' consumption worldwide. Antineoplastic agents (AAs), whether individually or in combination, are widely used in developed countries for cancer treatments. Subsequently, AAs have been quantified in environmental samples, mainly due to their poor removal by wastewater treatment plants, thus expecting the effects to the environmental health to be adverse, even at very low doses.

In this study, to understand the potential cytotoxic effects of AAs, the single and joint effects of three AAs with different mechanisms of action (trabectedin, doxorubicin, and oxaliplatin) were evaluated. For that, the zebrafish liver (ZFL) cell line was used as a model to test the hypothesis that AAs exhibit an unspecified cytotoxicity even at low doses. Cells were incubated using a wide range of increasing concentrations of AAs along with the control treatments for 72 h. The viability of cells exposed was determined by a fluorometric assay using resazurin (commercially available as PrestoBlue®), and morphological changes were observed and assessed by microscopy. Then, the IC50 values calculated for each AA were transformed into Toxic Units (TU) and a full factorial design was used to predict their joint toxicity pattern. The MIXTOX model was used to predict their joint toxicity using as starting point the reference models of Concentration Addition and Independent Action. Our results showed that the exposure to the three AAs in single exposures induced a reduction in ZFL cells viability in a concentration- and time-dependent manner, and it was observed that the most toxic was trabectedin followed by doxorubicin and oxaliplatin. Moreover, at morphological level, treated cells showed relevant alterations. Regarding the joint exposures, our results indicate significant interactions between the tested AAs. Since little is known on the effect of AAs on aquatic organisms, our results demonstrate that ZFL cells provide a relevant and sensitive tool to screen the toxicity of environmental pollutants in the frame of hazard assessment, highlighting the importance of investigating the potential of cell lines to serve as proxy to *in vivo* for the single and joint toxicity assessment of AAs.

## 1.02 Applications of Computational Toxicology in Environmental Risk Assessment

### 1.02.P-Mo001 Expanding SeqAPASS Capabilities to Protein Structural Comparisons Across Species

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The availability of protein structures has expanded significantly with advances in artificial intelligence. Specifically, AlphaFold (<https://alphafold.ebi.ac.uk/>) and MetaAI ESM Metagenomic Atlas (<https://esmatlas.com/>), which house the structures for hundreds of millions of AI predicted proteins from the diversity of species. The Protein Data Bank (PDB; <https://www.rcsb.org/>) contains empirically derived protein structures and other approaches, such as Iterative Threading ASSEmbly Refinement (I-TASSER; (<https://zhanggroup.org/I-TASSER/>)), that provide means for additional protein structure predictions. With these advances in predicting protein structures comes the opportunity to enhance the ability to compare species at the molecular level. Such comparisons lead to understanding similarities and differences across species that can improve understanding of chemical-protein interactions and conserved biological pathways. Protein sequence comparisons for conservation across species have been used as the primary evaluation in the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS; <https://seqapass.epa.gov/seqapass/>) tool for predictions of chemical susceptibility and pathway conservation. However, the intent of the SeqAPASS developers is to take advantage of all existing protein information as it becomes available and tools become more amenable to automation for predicting cross-species susceptibility. Therefore, SeqAPASS version 7.0 includes new features integrated in the interface to generate and capture protein structural models from the sequences aligned in Level 1 (primary amino acid sequence alignment), compare protein structures across species for an additional line of evidence toward protein conservation, and provide users with the protein structures for more advanced molecular docking, virtual screening, and possibly molecular dynamic simulation. Moving beyond protein sequence-based predictions to structure using the SeqAPASS web-based pipeline has led to enhanced understanding of the likelihood for chemical interactions, allowing advanced users the ability to examine binding affinities. This presentation will describe the new features of SeqAPASS v7.0 and demonstrate the utility of the enhanced features. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

### 1.02.P-Mo002 Exploring the Potential of *in silico* Machine Learning Tools for the Prediction of Acute *Daphnia magna* Nanotoxicity

**Surendra Balraadsing, Willie Peijnenburg and Martina G. Vijver, Leiden University, Leiden, Netherlands**

Engineered nanomaterials (ENMs) are ubiquitous nowadays, finding their application in different fields of technology and various consumer products. Virtually any chemical can be manipulated at the nano-scale to display unique characteristics which makes them appealing over larger sized materials. As the production and development of ENMs have increased considerably over time, so too have concerns regarding their adverse effects and environmental impacts. It is unfeasible to assess the risks associated with every single ENM through *in vivo* or *in vitro* experiments. As an alternative, *in silico* methods can be employed to evaluate ENMs. To perform such an evaluation, we collected data from databases and literature to create classification models based on machine learning algorithms in accordance with the principles laid out by the OECD for the creation of QSARs. The aim was to investigate the performance of various machine learning algorithms toward predicting a well-defined *in vivo* toxicity endpoint (*Daphnia magna* immobilization) and also to identify which features are important drivers of *D. magna in vivo* nanotoxicity. Results indicated highly comparable model performance between all algorithms and predictive performance exceeding ~0.7 for all evaluated metrics (e.g., accuracy, sensitivity, specificity, balanced accuracy, Matthews correlation coefficient, area under the receiver operator characteristic curve). The random forest, artificial neural network, and k-nearest neighbor models displayed the best performance but this was only marginally better compared to the other models. Furthermore, the variable importance analysis indicated that molecular descriptors and physicochemical properties were generally important within most models, while features related to the exposure conditions produced slightly conflicting results. Lastly, results also indicate that reliable and robust machine learning models can be generated for *in vivo* endpoints with smaller datasets.

## 1.02.P-Mo003 Predictive Modelling of the Maternal Transfer of Organic Pollutants to Next Generations in Reptile Species

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Maternal transfer of pollutants between mothers and their offspring presents one of the first exposure routes for newborn organisms before they themselves come into contact with the external environment, and this during sensitive early life stages where many fundamental development and organization processes take place. To support exposure assessment of early life stages, we aimed to develop a predictive model regarding the maternal transfer of organic pollutants that is applicable across reptile species. We developed a Bayesian orthogonal regression model to characterize the relationship between pollutant concentrations in mother and offspring tissues as dependent variables which are both measured quantities. The model explicitly accounts for uncertainty in measured variables by applying a Gaussian error distribution on both variables and constraining their range in relation to the population mean. Parameter estimation using Bayesian inference allowed the full parameter uncertainty to be propagated into the probabilistic model predictions. To support model development, we conducted a systematic search regarding measurements of organic pollutants in coupled samples from mother and offspring tissues. Subsequent data curation, including homogenization, standardization, and integration, resulted in a large, compiled database containing heterogeneous records from diverse species, diverse tissues, and diverse chemicals (including legacy POP such as PCB and OCP as well as newer compounds such as plasticizers). Alternative model formulations and extensions were developed to identify the most parsimonious model that can capture the influence of the diversity in species and chemical structures within the database, as well as account for the large proportion of censored data (records where measured concentrations were below the detection limits of the analytical instruments). Model goodness of fit was evaluated against the WAIC criterion and predictive performance assessed via cross-validation. The resulting model allows scientists and decision-makers to quantitatively link internal body burdens of mothers to subsequent generations in reptile species, a group which is underrepresented in ecotoxicology and chemical risk assessment.

## 1.02.T-01 MechoA+: A Significant Update to the MechoA Classification Scheme

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Knowledge of the mechanisms by which substances induce adverse effects is key to understanding, predicting, and limiting the impact of chemicals on humans and ecosystems. This is often based on the adverse outcome pathway blueprint of molecular initiating events (MIE) and some subsequent key events for a wide domain of species. Different schemes for attributing mode and mechanism of toxic action to chemicals have been developed historically but the most recent are the MechoA (Mechanisms of Toxic Action) and Sapounidou schemes. The improvement and subsequent merger of both schemes were carried out by KREATiS in collaboration with Liverpool John Moores University and supported by Unilever giving rise to a new scheme called MechoA+. This new tool was developed by a team of chemists, biologists and (eco)toxicologists using a holistic approach. It considers the mechanisms and effects observed in different species and experimental conditions to validate or invalidate the proposed MechoA subclass. Furthermore, protein orthology databases were used and the number of alerts covered by the scheme doubled. Thus, the spectrum of application of the model is greatly extended compared to previous schemes. It now covers many regulatory endpoints in toxicology and ecotoxicology. To boost its usage for regulatory purposes, including QSAR building and read-across, it will be made freely accessible in the OECD QSAR Toolbox as the “MechoA+” profiler, as is already the case for the current version, the iSafeRat® MechoA profiler. Another more advanced version of MechoA+, named MechoA Premium, available in iSafeRat® Desktop, is under development by KREATiS, and will be frequently updated to integrate new knowledge. These new advances will pave the way toward a broader and more versatile tool for regulatory use or in other areas such as R&D molecular design and eco-conception.

## 1.02.T-02 Explainable Deep Learning to Predict Bioaccumulation in Fish

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The rapid evolution of machine learning and explainable artificial intelligence (XAI) methods opened up new opportunities for predictive modelling in the context of molecular design and safety assessments. We explored explainable deep learning methods to predict bioaccumulation in fish from molecular structures. Bioaccumulation is an important parameter in the environmental risk assessment of chemicals and new alternative methods are required to reduce vertebrate testing. Despite limited numbers of tested molecules and the complexity of biological systems, artificial intelligence can be useful, especially in combination with human expert mechanistic knowledge.

We developed a new multitask deep learning model for the bioconcentration factor (BCF). The model is based on a continuous data-driven representation of chemical space that operates on simplified molecular-input line-entry system (SMILES) strings. In addition to BCF data, we utilized a large dataset for lipophilicity (logD) to improve model performance and extend the applicability domain. Our model is more accurate than previously published models (RMSE of 0.59 log units on an independent test set) and generalizes well to unknown regions of the chemical space. It is purely data-driven and therefore independent from expert-crafted rules or the definition of functional groups.

Moreover, we developed an explainability approach that provides valuable feedback to the user by highlighting the most influential parts of the molecule (SMILES characters). This additional explainability layer helped during model development, can foster acceptance of the predictions by allowing users to explore the inner workings of the model, supports the design of safe molecules, and can improve mechanistic understanding of the processes involved in bioaccumulation.

Overall, with its accuracy, the broad applicability, its ability to generalize, and the explainability aspects, our model satisfies the criteria for many use cases and could be considered an alternative for animal testing.

#### **1.02.T-03 EAS-E Suite for Chemical Safety and Sustainability**

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Thousands of new and existing chemicals require environmental risk assessment and the necessary information is quite limited or nonexistent for most chemicals. The freely accessible on-line Exposure And Safety Estimation (EAS-E) Suite platform includes curated, measured physical-chemical properties, environmental biodegradation half-lives, biotransformation half-lives, and production volumes for thousands of chemicals, quantitative structure activity relationships (QSARs) for predicting chemical information (if measured data are unavailable), and tools to aid chemical assessment decision-making. The EAS-E Suite includes various multimedia mass-balance environmental fate, bioaccumulation, and exposure models for a wide range of ecological receptors (plants, invertebrates, fish, birds, and mammals) as well as humans. The EAS-E Suite also includes the CiP-CAFÉ model for estimating life cycle chemical mode-of-entry and emission rates. The PROduction-To-EXposure High Throughput (PROTEX-HT) model combines CiP-CAFÉ and the RAIDAR, and RAIDAR-ICE models for simulating aggregate exposures to representative ecological receptors and humans in a one health approach for environmental risk assessment. Using only chemical structure as an input parameter, the EAS-E Suite also provides information for Persistence, Bioaccumulation, and Mobility assessments. The recently added internal Threshold for Toxicological Concern (iTTC) provides conservative safety-based screening information for existing chemicals as well as prospectively for new chemicals that have not yet entered the market. The objective of this study is to highlight the role EAS-E Suite can play in screening and prioritizing thousands of chemicals for hazard and risk assessment. The case study comprises a range of chemistries including flame retardants, plasticizers, biocides, personal care products, industrial chemicals, and chemical intermediates. The chemicals are compared for P<sub>OV</sub>, maximum BMFs (from aquatic and air-breathing organisms), and maximum exposures (mmol/kg-ww) in a range of ecological receptors, and safety. P<sub>OV</sub> ranges from about 10 h to 1,000,000 h. The maximum BMFs (i.e., fish, mammals, birds, humans) range from about 0.002 to 100. The maximum exposures span 12 orders of magnitude demonstrating the capacity to quickly screen and differentiate chemicals with high risk of exposures to ecological receptors. The simulations can be used to prioritize data needs and testing requirements to reduce unnecessary animal testing.

#### **1.02.T-04 MLinvitroTox: A Machine Learning-Based Hazard-Driven Prioritization for Environmental High-Resolution Mass Spectrometry Analysis**

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High-resolution mass spectrometry (HRMS) followed by nontarget screening (NTS) workflow for converting thousands of molecular “features” into quantified chemical structures is a cornerstone tool for comprehensive mapping of global pollution. However, since a complete elucidation of a large number of HRMS signals is not yet feasible, prioritization of the “relevant” signals is a crucial step. While the traditional HRMS prioritization approaches are often based on signals’ abundance or frequency underlining environmental exposures, they lack toxicological relevance critical for risk assessment. In this work, we propose to replace the traditional prioritization during NTS-HRMS/MS with a hazard-driven prioritization based on the unknown features’ potential toxicity, focusing the analysis on the signals with the highest potential to cause harm. For the prediction of features’ hazards, a machine learning tool (MLinvitroTox) was developed as a collection of optimized supervised classification models trained to predict hundreds of molecular toxicity endpoints from the high-throughput invitroDB (Tox21 and ToxCast) from the USEPA. When deployed on environmental data, MLinvitroTox takes molecular fingerprints of unknown HRMS/MS features as input (obtained from MSMS spectra via SIRIUS’s CSI:FingerID) and outputs their “toxicity fingerprints,” binary strings of 0’s and 1’s indicating their toxic activity in single endpoints. The results have shown that MLinvitroTox performs well in modeling, under validation, and with environmental samples. With optimal settings (XGB modeling algorithm, CSI:FingerID fingerprint [used in SIRIUS], smote oversampling of the minority class, repeated\_cv as resampling), average sensitivity values (toxicity discovery rates) were > 0.95 for a third (28 %) of the molecular toxicity endpoints and the majority of the known mechanistic targets (70 %). Deployment of MLinvitroTox on MassBank spectra (validation) and environmental samples (testing) showed that MLinvitroTox not only performed well in modeling, but also worked with pure spectra (sensitivity > 0.85) and real-life HRMS/MS raw data. MLinvitroTox can be tailored to various applications, e.g., support of effect-directed analysis of digitally frozen samples or an in situ hazard-driven prioritization for a rapid chemical risk assessment in an early warning system.

#### **1.02.P Applications of Computational Toxicology in Environmental Risk Assessment**

##### **1.02.P-Mo004 Improving Environmental Risk Assessment with Data Literacy and the Bayesian Regression Toolbox**

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From a data analysis perspective, environmental risk assessments (ERAs) are highly dependent on their domain context and data availability. The collection of appropriate data for an ERA can be challenging, as a variety of experimental and synthetic data are available, often with varying degrees of reliability. Identification of appropriate data sources is important, especially because expert curation of reliable data can be time and resource consuming.

For high quality ERAs, the understanding of the collected data is therefore crucial, especially the data-generating processes. Other important considerations are variations between experiments, incomplete data sets, or measurement limits. The evaluation of this information is important for choosing an appropriate modelling strategy, and, in the case of a Bayesian data analysis, the construction of meaningful priors.

Bayesian regression models (BRMs) offer great flexibility to address these challenges and can quantify uncertainties. Outside the Gaussian default, more appropriate probability distributions are available for count, survival, or concentration data. Information such as weights (reliability scores), censoring (values below measurement limits), or truncation (known lower or upper limits of a response variable) can also be included. This ensures that the data can be used “as is,” without subjective data manipulation. More complex variations are possible, for example by accounting for nonlinearity or by adding a hierarchical component (“random effects”).

A good understanding of BRM results is necessary to evaluate the quality of the model, as well as to draw the right conclusions of the posterior model results for an ERA. This includes both the understanding, but also the visualization and communication of the results and their uncertainties.

The above points can be collectively described as data literacy. A stronger focus on this concept, together with BRMs, has the potential to greatly improve the quality of ERAs under regulatory frameworks, as well as improve a shared understanding of the involved stakeholders.

#### **1.02.P-Mo005 A Bayesian Network Tool for Predicting Fish Acute Toxicity Based on Fish Embryo Toxicity Test Data: Optimizing Weights of Evidence**

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Fish Embryo Toxicity (FET) testing has been proposed as an alternative to using juvenile or adult fish in acute toxicity testing, to reduce the number of live animals required for hazard assessments of chemicals. The European Chemicals Agency has recommended the development of a Weight-of-Evidence (WoE) approach for strengthening the evidence from FET testing. While WoE approaches in the past have been largely qualitative, we have developed a Bayesian network (BN) model for using FET data in a quantitative and probabilistic WoE approach. The purpose of this BN model is to predict fish acute toxicity of a given chemical from data on fish embryo toxicity in combination with other types of available information (lines of evidence): quantitative structure-activity relationships (QSARs), toxicity to other species (algae and daphnids), and fish gill cytotoxicity. The tool is publicly available through a web-based user interface at <https://swift.hugin.com/models/FET>, and demonstrations of this tool can be provided upon demand. In previous presentations from this project, we have explored different approaches for estimating the weights for each line of evidence, as well as approaches to evaluating the model performance.

This presentation will focus on more advanced methods for optimizing the weights for the lines of evidence: the use of machine learning algorithms (i.e., the Expectation Maximization algorithm for parameter estimation from data) for estimating conditional probability tables. We will address the general problem of estimating a high number of “probability parameters” (entries in the conditional probability tables) from a limited number of cases (toxicity values). While the purpose of this model is to predict acute fish toxicity from fish embryo toxicity, our solutions can be relevant more generally for evaluating animal alternatives in regulatory toxicity testing, or even more generally for other types of environmental assessments.

#### **1.02.P-Mo006 Computational Tools for the Development and Application of QSARs for the Estimation of Multiple Endpoints**

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In this work we present several computational tools recently developed at the University of Insubria to model and estimate properties of interest for the risk assessment of chemicals on the basis of Quantitative Structure Activity Relationships (QSARs). The software, i.e., IVBP-Suite, QSAR-ME Profiler, and Chemo-MBUILDER, can run on platforms supporting the Java™ runtime (e.g., Windows™, Mac OS™ and Linux).

IVBP-Suite is dedicated to the estimation of the *in vitro* intrinsic clearance of chemicals by running QSARs based on experimental data measured in human, rat, and mouse. These models were developed following reactivity pathways based on putative cytochrome P450 (CYP) mediated reactions. To automate the selection of the pertinent QSARs the Smart-Cyp ToxTree plugin is automatically run.

QSAR-ME Profiler applies models for the prediction of endpoints characterizing properties, activities and fate of organic chemicals such as, for example, acute toxicity in multiple organisms, bioconcentration and biomagnification in fish, biotransformation (*in vivo* and *in vitro*), and a PBT profiler. QSAR-ME Profiler includes functionalities for the study of structural similarity among molecules used to develop the QSARs, or new molecules profiled by the user. The molecular structures of chemicals of interest can be depicted and compared with the automatically detected most similar chemicals. QSAR-ME Profiler



includes sets of QSARs developed using Multiple Linear Regression (MLR) or Linear Discriminant Analysis (LDA) and validated according to OECD guidelines. Both IVBP-Suite and QSAR-ME Profiler automatically run PaDEL-Descriptor software for the calculation of the molecular descriptors, if not provided by the user.

Finally, the Chemo-MBuilder software streamlines QSARs development including overfitting control in variable selection and the evaluation of coincidental relationships between the descriptors and the endpoint, with a focus on calculation speed performances. The three software programs are designed to be user-friendly following simple workflows. Batch results are provided as tables, and the analysis of predictions is supported by the graphical user interface. IVBP-Suite and QSAR-ME Profiler are aimed to streamline, organize, and simplify the application of batteries of QSARs, which is particularly useful for their regulatory application, and are freely available from the internet (<http://dunant.dista.uninsubria.it/qsar/>). Chemo-MBuilder is currently under development.

#### 1.02.P-Mo007 A Benchmark Dataset for Machine Learning in Ecotoxicology

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The application of machine learning methods to ecotoxicology is still in its infancy. Model performance is highly dependent on the features and the quality of the data set on which model training and testing are performed. Comparing model performances across studies done on different data sets is practically impossible, since data sets are different in number/kind of species and chemicals. Data set curation requires biological expertise, which might pose a barrier of entry for machine learning experts without the necessary background. To remove this barrier and to attract researchers from outside our field to apply their expertise to ecotoxicological problems, we, a team of ecotoxicologists and machine learning experts, provide an expert-curated and well-characterized data set on acute mortality and related endpoints in the three taxonomic groups of fish, crustaceans, and algae. We describe the process of feature selection and data cleaning. Apart from the core data set of ecotoxicological effect data, we expanded the data set with supplemental species-specific and chemical information. Based on the assumption that closely related species will react to chemical exposure more similarly than unrelated species, we included a phylogenetic distance matrix. Where available, we included ecological and life-history data. Chemical information is supplemented by chemical properties as well as different molecular representations for modeling. We discuss potential pitfalls regarding train-test splitting of such a complex data set to avoid data leakage. We present insights and patterns that can be deduced from the raw data alone, such as species sensitivity distributions (SSD). Additionally, we propose concrete tasks to the community that should be answered based on the data set and could help answer pressing questions from our field. These include predictions across taxonomic groups and the identification of potential surrogate species.

#### 1.02.P-Mo008 Challenges with QSAR Models for Chemical Risk Assessment —

##### Comparing Empirical Data with Predictions from Three QSAR Platforms Across Six Endpoints

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The total number of chemicals on the world market continues to increase, and toxicity testing is struggling to keep up. An important element of chemical risk assessment and, by extension, chemical regulation, is trustworthy toxicity estimations of the chemicals in question. *In silico* tools, such as ecotoxicity quantitative structure-activity relationship (QSAR) models, are being used to fill data gaps when empirical data are missing, but to what extent can we trust these predictions? In this paper, three freely available ecotoxicity QSAR platforms (ECOSAR, Vega and the Toxicity Estimation Software Tool - T.E.S.T.) have been tested against empirical data from the United States Environmental Protection Agency (USEPA) ECOTOX database and the European Food Safety Authority (EFSA), to determine performance for six common endpoints: acute and chronic toxicity for algae, daphnia, and fish. The empirical databases have been filtered based on OECD guidelines and QSAR endpoints, and for every chemical with data for at least one endpoint present, QSAR predictions have been generated. Each platform contains multiple QSAR models, so different approaches to gather the predictions in a single value per platform, substance, and endpoint were implemented. A number of different scenarios, based on handling of endpoint filters, pKa considerations, logP ranges, and platform processing methods, were set up and systematically explored to identify confounding factors or other attributes that might impact prediction quality. Finally, predictions were compared to empirical data to estimate platform performance under different scenarios. Initial results indicate that predictions from all platforms correlate well with empirical data for acute daphnia and fish endpoints, but for fish and algae chronic endpoints, there are issues with applicability domain and/or performance for both ECOSAR and Vega. Given that the results hold, caution is advised when using chronic QSAR predictions in chemical risk assessment and regulation.

#### 1.02.P-Mo009 Tiered Methods for Screening-Level Ecological Hazard and Risk Assessment: Case Study Application to Octamethylcyclotetrasiloxane, D4

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Thousands of chemicals are undergoing hazard, exposure, and risk assessment using hazard- and risk-based methods in various regulatory programs worldwide. Hazard-based approaches compare a chemical's persistence (P), bioaccumulation (B), and toxicity (T) to multiple bright-line "pass/fail" categorization criteria. Risk-based approaches quantitatively compare measured or

predicted exposure estimates to a toxicological effect threshold such as an environmental (no) effect concentration ((NO)EC) or a chemical intake rate threshold (IRT). In the case of inadequate or missing monitoring data, exposure models can be used or integrated with available data to address data gaps and better characterize uncertainty in exposure estimation. They can also provide valuable guidance for chemical prioritization or, alternatively, de-prioritization of chemicals. In the present study, we show that the PROduction-To-EXposure High Throughput (PROTEX-HT) model, the Chemicals in Products - Comprehensive Anthropospheric Fate Estimation (CiP-CAFE) model, and the Risk Assessment Identification And Ranking (RAIDAR) model can be used in a tiered approach with temporally and spatially relevant production/import volumes and applicable functional use category information to estimate hazard, exposure, and risk for chemicals. We apply the RAIDAR model parameterized as the European regional environment (RAIDAR-EU) to a cyclic volatile methyl siloxane (cVMS), octamethylcyclotetrasiloxane (D4) as a case study. Tiered methods for estimating releases to the environment progress from conservative assumptions to more realistic assumptions, i.e., using CiP-CAFE. The model predictions at each Tier are compared with monitoring data. Tiers 2 and 3 show similar results indicating that CiP-CAFE can be used to predict release of chemicals such as D4. Additionally, literature toxicological exposure and intake endpoints are used to estimate potential ecological risk for various aquatic and terrestrial organisms. Our work illustrates that by using temporally and spatially relevant production and import volumes and use information for D4 in Europe, fate and food web concentrations can be reliably predicted. The work highlights how PBT assessment and exposure and risk estimation can be used together for more comprehensive chemical assessments. Finally, this process can be used to focus research to fill relevant data gaps and reduce animal testing.

#### 1.02.P-Mo010 Predictive QSAR Models to Estimate the Toxic Potential of Pesticide's Metabolites to Honeybees

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The metabolism of pesticides leads to changes in their molecular structure which may be sometimes beneficial or give as result toxic metabolites that may affect nontarget organisms such as honeybees. The estimation of the toxicity of metabolites has not been explored extensively by scientists, while it is an important part in pesticides or drug development to identify the possible mechanism of metabolism and their toxic or adverse effects. Experimental toxicity assessment for a large number of metabolites is a costly measure in terms of time, money, and number of sacrificed animals. In this background experimental toxicity data (NOEL) measured in honeybees for 26 metabolites of pesticides have been collected from the OPP-Ecotox database and subjected to QSAR model development. The model development was done by using multiple linear regressions (MLR) and genetic algorithm (GA) for feature selection. The 2D theoretical molecular descriptors were calculated using the PaDEL descriptor software. Further, the leverage approach was used to assess the applicability domain (AD) of the models and to check the reliability of predictions. All the QSARs have rewardable performances –valuated by internal ( $Q^2_{LOO} = 0.590 - 0.726$ ,  $R^2_{adj} = 0.777 - 0.798$ ) and external validation ( $Q^2F_n = 0.741 - 0.870$ ,  $R^2M_{avg} = 0.560 - 0.758$ ,  $CCC_{ext} = 0.840 - 0.896$ ) which assured the validity and the generalizability of these models within their applicability domain. Hence the outcomes of this study will be helpful for researchers to predict and screen the toxicity of metabolites to honeybees in the initial stage of development of parent compounds. Furthermore, they can provide information useful for the design of alternatives to parent pesticides leading to toxic metabolites, or, more in general, to support the risk assessment of pesticides.

#### 1.02.P-Mo011 Machine Learning for Toxicity Categorization from Molecular Descriptors: An Alternative to Conventional QSAR Models

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Hundreds of thousands of chemicals have been registered for production and use worldwide and a significant fraction of those will eventually make their way into the environment. However, for most of these chemicals, knowledge on environmental fate (persistence, mobility) and toxicity is largely missing, and filling those gaps with experimental data is practically unfeasible for such a large number of substances. Therefore, models to predict hazard indicators for chemical prioritization and risk assessment play an increasingly important role and the reliance on such models requires continuous reevaluation and improvement of existing approaches. Most existing modeling strategies are known as quantitative structure activity relationship (QSAR) models. These rely on building linear and/or nonlinear relationships between the structural descriptors of the chemicals and the modeled activity/property. However, these models are often built on very similar chemical classes (i.e., homogeneous training sets) and are limited in terms of the number of chemicals evaluated. As a consequence, many chemicals fall outside of the applicability domain of the QSAR models and the models are often used in extrapolation mode when used for prediction, which may result in large prediction errors.

Here we present an alternative strategy to QSAR models for chemical prioritization, on the example of acute aquatic toxicity as a predicted endpoint. We developed a machine learning-based direct classification strategy, where molecular descriptors are directly converted into toxicity categories, without a separate (and error-prone) prediction of the chemical activity (here: 96h lethal concentration, LC50). As a proof-of-concept our model was trained and tested with experimentally derived 96 h LC50 values for acute fish toxicity for 907 organic chemicals. The model was then applied to predict toxicity categories for 32 000 chemicals from the NORMAN Substance Database (Norman SusDat). Compared to a conventional QSAR model based on the same training and test set, our direct classification model showed a five-fold decrease in wrong categorization. Our model can be applied both to data-driven toxicity categories (e.g., derived via k-means clustering from a given dataset) or to fixed regulatory derived categories (e.g., from the Globally Harmonized System of Classification and Labelling of Chemicals [GHS]) and represents an important step toward a more data-driven environmental risk assessment.

### 1.02.P-Mo012 Addressing Uncertainty in Chemical Partitioning Properties and Prospects for Improvement

**Trevor N Brown, PhD**, Jon A. Arnot and Alessandro Sangion, Arnot Research and Consulting Inc. (ARC), Canada

Physical-chemical properties are a fundamental aspect of environmental toxicology and chemistry, and chemical evaluations. These properties are key determinants of chemical fate in the environment, in organisms, and in experimental test systems. Properties such as water solubility (WS), vapor pressure (VP), octanol-water partition ratio ( $K_{OW}$ ), octanol-air partition ratio ( $K_{OA}$ ), and air-water partition ratio ( $K_{AW}$ ) are among the most commonly required data for understanding chemical fate and distribution in natural and artificial systems, and for determining the potential for adverse effects to humans and the environment. Compared to the vast number of chemicals produced and used by society, relatively few have complete high quality measurements of these fundamental properties. For many chemicals (e.g., those with low VP, low WS) technical methods are not adequate to measure these properties. Given the critically important nature of these chemical properties and significant data gaps there is a need to develop and validate reliable methods for their prediction. Uncertainty and applicability domain (AD) are vital considerations when considering measured or predicted properties to inform decision making. Quantitative Structure Property Relationships (QSPRs) and other estimation methods have been developed and applied for decades. Poly-parameter Linear Free Energy Relationships (PPLFERs) are also established tools for predicting physical-chemical properties of chemicals. Poly-parameter Linear Free Energy Relationships combine system parameters, that describe the system properties, and solute descriptors, that depend on the chemical. Here we describe the development and validation of a new suite of predictive tools using the Iterative Fragment Selection (IFS) methods. The IFSQSARs provide explicit estimates of AD and uncertainty with predictions. We describe the general approach to developing and validating the IFSQSARs and compare their performance against other commonly used QSPRs (e.g., USEPA's EPI Suite, OPERA models) and against empirical data. To facilitate applications and comparisons of the new IFSQSARs they have been implemented in the freely available on-line Exposure And Safety Estimation (EAS-E) Suite platform ([www.eas-e-suite.com](http://www.eas-e-suite.com)). These QSPRs have been integrated into the EAS-E Suite platform, which allows for the prediction of  $K_{OW}$ ,  $K_{OA}$ ,  $K_{AW}$ , VP, and WS which can then be used to parameterize the various fate, toxicokinetic, bioaccumulation, exposure, and risk models in EAS-E Suite.

### 1.02.P-Mo013 iSafeRat® Platform: Development and Improvement of Mechanistic High Accuracy-QSAR Models to Predict Acute and Chronic Aquatic Toxicity

**Floriane Larras** and Paul Thomas, KREATiS, France

Quantitative Structure Activity Relationship models are relevant alternative methods in response to the need to reduce testing on vertebrates, time to market, and cost compared to standard methods. The iSafeRat® platform, developed by the (eco)toxicologists and chemists of KREATiS, aims, among others, to provide reliable predictions of chemical acute and chronic toxicity to fish, daphnia, and algae, and also Activated Sludge Respiration Inhibition Test, considering their mechanism(s) of action (MechoA). So far, 30 High Accuracy-QSAR models have been created, mostly dedicated to acute toxicity predictions for multiple mechanisms of action and thus a wide variety of structural groups (e.g., MechoA 1.1, membrane destabilisers such as alkyls, alcohols, ketones; MechoA 2.1, enzymatic hydrolysis, e.g., esters, amides; MechoA 3.1, reactive substances, e.g., aldehydes, epoxides, etc.). However, the development of aquatic chronic toxicity QSAR models for these MechoA has also been a high priority. For example, chronic toxicity data can be already required by REACH for substances manufactured or imported in quantities of 100 tonnes or more per year and are often the subject of ECHA draft decisions. Thus, while recent updates to iSafeRat® accounted for the improvement of the applicability domain of existing models (e.g., including new chemical structures such as phosphates), emphasis have been placed on the development of new models for chronic toxicity. The development of such models is a challenging task as high-quality chronic toxicity data are scarce, especially for organisms like fish and also for particular chemical functions. But more than generating data, the development of chronic models also supported the mechanistic understanding of chemical toxicity to aquatic organisms. Indeed, the comparison of acute and chronic regressions for the same biological compartment and the same mechanism of action unraveled potential hints to further investigate. The poster will include details of the validity check for new iSafeRat® models.

### 1.02.P-Mo014 Development of an *in silico* Structural Profiler Facilitating Mechanistically Grounded Classification of Aquatic Toxicants

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When considering environmental safety assessment of chemicals within regular industrial use, it is acknowledged that data gaps in many instances exist. The adoption of predictive (*in silico*) toxicological methodologies, spanning QSAR and structural rule-based systems, has been advanced as practical means by which this issue might be addressed. Amongst these techniques, the chemical classification and mode of action assignment schemes devised by Verhaar et al. (1992) and Russom et al. (1997) have achieved particular prominence. Despite their utility, such profilers are by now decades old: their coverage of chemical space and taxa is restricted, and the resolution offered in terms of mechanistic detail limited.

As such, we have sought development of an updated scheme—expanding the breadth both of chemical and taxonomical coverage relative to its forebears. Further advancement was offered in terms of characterisation of toxic mechanism—which took advantage of improved knowledge of molecular initiating events (MIE), and thus provided a link to the broader framework of adverse outcome pathways. The novel scheme was structured such that screened compounds were granted progressively finer grains of mechanistic classification, extending from highest tier (reactive domain: narcosis, nonspecific chemical reactivity, specific

biological interaction) through grouping (10 being present: e.g. mitochondrial disruption) and subgrouping (totalling 25: e.g., specific impairment of electron transport chain component) on to MIE (a sum of 60: e.g., inhibition of succinate dehydrogenase). Formal representation of MIE was achieved through derivation of structural alerts (183 in total) encoded as SMILES arbitrary target specification (SMARTS).

The implementation of this rule-set as a practical, freely accessible profiling tool was realised through construction of a workflow within KNIME analytical software, employing the SMARTS strings as its basis (accessible from [www.doi.org/10.5281/zenodo.7100972](https://www.doi.org/10.5281/zenodo.7100972)). Screening of representative compound inventories revealed superior coverage relative to Verhaar and Russom schemes, as regards both quantity of substances classified and applicable phyla/species recognised. Integration with the existing KREATiS MechoA rule-set is further proposed as means through which present shortcomings may be efficiently addressed.

#### **1.02.P-Mo015 Comparison of the Performance and Domains of *in silico* Schemes to Classify Environmental Chemicals**

**Mark Cronin<sup>1</sup>, James Firman<sup>2</sup>, Franklin Bauer<sup>3</sup>, Geoffrey Hodges<sup>4</sup>, Bruno Campos<sup>4</sup>, Jayne Roberts<sup>4</sup>, Steve Gutsell<sup>4</sup>, Paul Thomas<sup>3</sup> and Mark A. Bonnell<sup>5</sup>,** (1)Liverpool John Moores University, United Kingdom, (2)School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, United Kingdom, (3)KREATiS, France, (4)Unilever - Safety and Environmental Assurance Centre (SEAC), United Kingdom, (5)Environment and Climate Change Canada, Canada  
*In silico* classification schemes are commonly applied to assist in grouping of chemicals ahead of read-across and the application of quantitative structure-activity relationships (QSARs). Chemistry-based knowledge has been used in this way for over 30 years, since the publication of the Verhaar scheme. These tools, which can be considered to be *in silico* New Approach Methodologies (NAMs), assist greatly in data gap filling for regulatory and other purposes. This investigation compared the performance and potential applicability domains of four schemes, with particular reference to a new classification system. The schemes considered were those of Verhaar, Russom, MechoA (Bauer) and, most recently, Sapounidou. *In silico* implementations of each were assessed by screening a test inventory of approximately 5500 common pollutants. In terms of profiling, all schemes are based on (slightly) different approaches, with the Sapounidou scheme including information from putative molecular initiating events to facilitate classification. MechoA was found to assign the greatest proportion of compounds to particular mechanisms, although the Sapounidou scheme has more information on the specific and reactive mechanisms of action. An in-depth analysis of the Sapounidou profiler was performed, screening over 70 000 structures from distinct inventories representing REACH chemicals, pharmaceuticals, cosmetics ingredients, botanical natural products, and pesticides. Whilst coverage of some inventories was good, further information to classify pharmaceuticals is required. Other areas where the domains of all profilers could be improved is in strengthening the identification of narcotics, to enhance confidence in this classification (some schemes simply default to narcosis should no specific mechanism /mode be assigned).

#### **1.02.P-Mo016 A Comprehensive Similarity Approach to Identify Potential SVHC Chemicals**

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An automated method for screening and prioritization of chemicals is essential to ensure that available evaluation capacity is invested in those substances that are of highest concern. To facilitate the identification of substances of potential concern, we recently developed a structural similarity model that evaluates the structural similarity of substances with unknown properties to known Substances of Very High Concern (SVHC) [Wassenaar et al. 2022; <https://doi.org/10.1002/jcc.26859>]. Substances that are structurally similar to known SVHCs are selected for further evaluation, as a high resemblance in chemical structure could be an indication of comparable effects ("similar property principle").

Although this method works great at identifying chemicals that exhibit small structural differences with known SVHCs, it does not address the toxicokinetics and mode of action (MoA) of the chemicals. Extension of the ZZS similarity tool with other points of chemical and biological similarity can increase the confidence in the similarity predictions.

In this study we will investigate the extension of the chemical 'global' similarity to site specific similarity (i.e., structural alerts) and 'local' similarity (i.e., the chemical composition in close proximity to the relevant structural alerts). In addition, we investigate the benefits of adding a model that predicts biological similarity in the form of a physicochemical profile and bioassay-based profile.

By including various similarity descriptors our extended methodology is likely to result in an increased predictive performance. This will specifically result in improved predictions for chemicals that were previously identified as false positives which have a high overall ('global') similarity but are, e.g., lacking the structural alert causing the SVHC concern. In addition, improvements will likely be observed for previously identified false negatives which have a slightly larger difference in their (global) structure but are expected to result in a similar level of toxicity. Consequently, these model improvements could enhance the identification of potential SVHC chemicals.

The poster we intend to present will focus on the above-mentioned improvements regarding chemical and biological similarity based on preliminary findings.

#### **1.02.P-Mo017 Identification of Machine Learning Algorithms and Molecular Fingerprints for the Development of a Target-Specific Bioactivity Prediction Model Based on ToxCast Bioassay Data**

**Jaeseong Jeong, Su-Yong Bea and Jinhee Choi,** School of Environmental Engineering, University of Seoul, Seoul, Korea, Republic of (South)

Machine and deep learning approaches have been increasingly used in toxicology. The ToxCast program uses high-throughput screening (HTS) to screen biological activity to determine the potential toxic effects of environmental chemicals. It covers nearly 10K chemicals using 1473 *in vitro* bioassays. In this study, we identified the best model for 737 ToxCast assays to develop an effective bioactivity prediction model based on the ToxCast data. For each assay dataset, 30 models were trained by combining six algorithms (gradient boosting tree, random forest, multilayered perceptron network, k-nearest neighborhood, logistic regression, and naïve Bayes) and five molecular fingerprint representations of the chemical structure (MACCS, Morgan, layered, RDKit, and pattern). The synthetic minority oversampling technique (SMOTE) was used to balance the data. Of the 737 best models for each assay, 35 models with acceptable performance were selected based on the F1 score and accuracy. Finally, the selected models were analyzed according to the number and type of training data, and the type of algorithm and molecular fingerprints used for learning. It is important to utilize and study existing HTS data because biological activity data enable toxicity evaluation based on the mechanism of toxicity. We believe that our results can be used as a cornerstone for a wide range of *in silico* toxicity prediction studies.

#### **1.02.P-Mo018 Deconvoluting Chemical Mixtures in Aquatic Environment by Integrative High-Throughput Screening, Nontarget Analysis, and Artificial Intelligence**

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Ecosystems are often exposed to complex mixtures of chemicals. Identifying causative toxicants is essential to risk assessments but there are many data gaps among chemical occurrence and biological effects. Based on a novel event-driven taxonomy (EDT) framework that clusters chemical stressors by bioactivity, an artificial intelligence-assisted integrated testing strategy (ITS) was constructed for assessing aquatic risk by integrating high-throughput screening cell bioassays and chemical predictions. This EDT-based ITS was then evaluated using complex sediment mixtures eliciting aryl hydrocarbon receptor activation and oxidative stress response. While mixture prediction using the target analysis that was oriented by expert knowledge explained less than 10% of observed sediment bioactivity, a big data-driven suspect analysis based on text-mining expanded the fraction explained to >80%. Additionally, deep learning models were developed to extract the fingerprints of bioactive suspect candidates and convert these fingerprints to high resolution mass spectrometry-recognizable fragment ions for nontarget analysis using GC-qToF-MS. Overall, we present a mixture deconvolution ITS based on a novel model of “bioactivity-fingerprint-toxicant” to connect the patchy datasets through artificial intelligence.

#### **1.02.P-Mo019 *in-silico* Model-Based Exploration of the Importance of Gut Metabolism in Human Exposure and Toxicokinetic Modeling**

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Intestinal metabolic biotransformation or gut metabolism plays an important role in both the absorption and elimination of chemical contaminants: The preabsorptive intestinal and hepatic biotransformation, also known as the “first-pass effect,” reduces the absorption of ingested chemical contaminants into the systemic circulation, whereas postabsorptive hepatic and intestinal biotransformation reduces the human body burden of chemical contaminants by eliminating them from the systemic circulation. While *in silico* high-throughput physiologically based toxicokinetic (PBTK) models consider hepatic biotransformation, intestinal biotransformation, also known as gut metabolism, is often overlooked, and the relative importance of pre- and postabsorptive biotransformation is unclear. Experimental evidence has shown rapid intestinal biotransformation, e.g., with a half-life at a level of minutes for certain phthalate esters in metabolism by lipases. Neglecting intestinal biotransformation may result in a substantial overestimation of exposure to chemicals in high-throughput chemical screening. In this presentation, using an *in silico* PBTK model, we evaluate (i) the relative importance of pre- and postabsorptive biotransformation in controlling the presence of chemical contaminants in the human body, and (ii) important factors that govern such relative importance. Since *in vivo* biotransformation data are often inadequate for chemical contaminants, the model also includes an *in vitro* to *in vivo* extrapolation module to allow the use of *in vitro* data. The model shows that the relative importance of the rates of intestinal biotransformation and epithelium permeation greatly impacts intestinal absorption efficiency. In cases of phthalates where biotransformation occurs at a level of minutes, more than 60% of the ingested amount is biotransformed in the gastrointestinal tract before absorption. Overall, our work highlights the importance of intestinal biotransformation in human exposure and toxicokinetic modeling. This model also enables us to more accurately screen the absorption efficiency of chemicals through dietary and nondietary ingestion.

#### **1.02.P-Mo020 Considerations for Applying the Parallel Artificial Membrane Permeability Assay (PAMPA) in the Screening of Gastrointestinal Absorption of Chemicals of Environmental or Occupational Concerns**

**Shenghong Wang**, Zhizhen Zhang, Dingsheng Li and Li Li, School of Public Health, University of Nevada, Reno

Chemicals present in the environmental and occupational settings can exert adverse health effects on humans after gastrointestinal absorption into the systemic circulation. Parallel Artificial Membrane Permeability Assay (PAMPA) has gained advocacy for its application to measure the effective permeability toward pharmaceuticals. However, since chemicals of environmental or occupational concerns differ from pharmaceuticals in hydrophobicity and volatility, a thorough, mechanistic understanding of chemical mass transfer in PAMPA is warranted if we seek to expand the applicability of PAMPA. Here, we introduce an *in-silico* mass balance model, which describes chemical mass transfer in PAMPA based on inputs of fundamental physicochemical properties, e.g., molecular weight, partition coefficient, and dissociation constant. The model’s performance is evaluated by an

agreement between predicted and measured permeabilities of 1383 chemicals, which indicates that 95% of the estimated permeabilities either fall in the same order of magnitude or are more conservative than the measurements. The model predicts an inverted U-shaped dependence of permeability on the octanol-water partition coefficient ( $\log K_{OW}$  for neutral compounds and  $\log D_{OW}$  for ionizable compounds), with the maximum permeability occurring in  $\log K_{OW}$  or  $\log D_{OW}$  ranging between 0 and 2. The model estimates a high membrane retention rate for hydrophobic chemicals, as well as the loss of volatilization to the headspace of the PAMPA apparatus for highly volatile chemicals. Notably, the measured permeabilities of hydrophobic chemicals are remarkably sensitive to specific experimental conditions, e.g., frequency of stirring, and incubation time, making measurements under different conditions less comparable. More importantly, for highly hydrophobic chemicals ( $\log K_{OW}$  or  $\log D_{OW}$  greater than 3.8), steady-state mass transfer, which is the fundamental assumption of PAMPA, can never be achieved. Therefore, the current design of PAMPA needs to be modified before its application to chemicals of environmental or occupational concerns. Our work provides an *in-silico* mechanistic approach in support of efficiently and defensibly predicting the permeability of chemicals and complements the current laboratory approach.

#### 1.02.P-Mo021 Addressing Applicability Domain and Uncertainty in High-Throughput Toxicokinetic Data and Applications

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Quantitative knowledge of toxicokinetic (TK) processes is necessary to better understand relationships between external and internal exposures of chemicals. New Approach Methodologies (NAMs) such as *in vitro-in vivo* extrapolation (IVIVE) and high-throughput toxicokinetic (HTTK) modeling can be used to obtain TK data necessary for chemical priority setting, screening, and risk assessment. However, there is a need to systematically examine existing data and NAMs to foster confidence in their application for scientific evaluations and decision-making by establishing the applicability domains (AD) of existing HTTK models and databases. This case study determines the AD and assesses the uncertainty of current IVIVE and HTTK data and models to estimate administered equivalent doses (AEDs) from *in vitro* bioactivity test data.

Different *in silico* approaches are applied to calculate total elimination half-life ( $HL_T$ ) and steady-state blood concentration ( $C_{SS}$ ) to identify their AD, particularly relative to their required input parameters to better understand the chemical space that can be covered by current HTTK models. The comparison includes: i) Quantitative Structure Activity Relationship (QSAR) models to directly estimate  $HL_T$  and a composition model where tissue partitioning is a function of the composition of the tissue (i.e., storage lipid, membrane lipid, structural protein, serum albumin and water) to calculate volume of distribution ( $VD_{SS}$ ), ii) a generic one-compartment physiologically based toxicokinetic (1Co-PBTK) model that can be parameterized to different mammals as implemented in the Exposure And Safety Estimation (EAS-E) Suite platform, and iii) the “3 Compartment steady state” model developed by the USEPA as implemented in the “httk” R package and accounting for metabolism on the basis of *in vitro* biotransformation data. The results of this work generally support the computational IVIVE and HTTK methods required to estimate AEDs. General guidance and recommendations are provided for applying HTTK methods for AED calculations and improving HTTK modelling and risk estimation.

#### 1.02.P-Mo022 A Case Study on Skin Sensitization Assessment Using OECD QSAR Toolbox **Heekyung Bae<sup>1</sup>, Hyejin Kim<sup>1</sup>, Yeonjung Jung Park<sup>1</sup>, Jinseon Son<sup>1</sup>, Kwangsik Park<sup>2</sup>, Sun-Young Park<sup>3</sup>, Ok-Nam Bae<sup>4</sup>, Sanghee Lee<sup>3</sup> and Hyeonsoo Park<sup>1</sup>**

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The QSAR toolbox is a software application intended for the use of governments, the chemical industry, and other stakeholders in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The QSAR toolbox incorporates information and tools from various sources into a logical workflow and identification of relevant structural characteristics and potential mechanism or mode of action of a target chemical. The OECD is continuously updating the toolbox so that it can be used for the evaluation of chemicals such as EU REACH, and updating to ver. 4.5 in September 2021, Automated Workflow to implement OECD GL 497 that can predict skin sensitization and confirm results for Skin Sensitization for Defined Approach (DASSAW) function has been added. OECD GL 497 is expected to be actively used to evaluate skin hypersensitivity of chemicals without conducting animal experiments because it can derive reasonable prediction results for skin hypersensitivity by combining the DASS function of the QSAR toolbox with the 2o3 method. Therefore, in this study, among substances classified as skin sensitization in the National Chemical Information System (NCIS) in Korea, substances for which *in vitro* test data exist (positive) were selected as positive substances, and among substances not classified as skin sensitizers, *in vitro* test data were selected. Existing (negative) substances were selected as negative substances and the skin hypersensitivity prediction results using the DASS function of QSAR toolbox were confirmed. Combining the skin hypersensitivity prediction result using the DASS function of the QSAR toolbox with the 2o3 method, the final conclusion for skin hypersensitivity classification was confirmed according to the scoring method per OECD GL 497. Through this, the usability of the evaluation results according to OECD GL 497 as registration data for chemicals such as EU REACH and K-REACH was confirmed.

#### 1.02.P-Mo023 Behavioural Fingerprinting with Machine Learning to Characterise Micropollution in Wastewater Effluents

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Advances in technology and chemical understanding are highlighting the abundance and the diversity of micropollutants present in wastewater effluents. Biosurveillance methods based on locomotion behaviour tracking, such as ToxMate monitoring, have proved effective in capturing surges of micropollution in wastewater effluents, alerting operators to periods of potentially detrimental discharge. Whilst on-line alerting to micropollution is an essential step, the question remains: What is the contamination nature at the origin of water quality degradation? We hypothesized that the diversity of species behaviour response when exposed to contaminants can help to discriminate between exposure to different types of contamination. The objective of this poster presentation is to show how time series modelling, coupled with machine learning, may be the key to better contaminant identification thanks to behaviour fingerprints determination.

ToxMate biosurveillance apparatus uses videotracking to generate positional data ( $x,y$ ) at 10 frames per second for 16 organisms of three bio-model species of aquatic invertebrates: *Gammarus*, *Erpobdella*, and *Radix*. Statistical modelling of the resulting big-data time series allows the quantification of the studied biomarker: avoidance behaviour, which correlates in real time to sharp increases in micropollutant presence.

Time series data from ToxMate experiments, collected from laboratory exposure to more than 80 micropollutants, was studied using functional data analysis (FDA). Combining the signals in a multispecies behavioural tracking, multidimensional analysis (known as vector-valued FDA) was used to first model the data as instances of functional random variables using B-Spline approximations, and then use dimensional reduction techniques such as functional principal component analysis (FPCA) as well as ML algorithms such as K-Means clustering, K Nearest Neighbours, and Quadratic Discriminant Analysis classification methods to form groups of behavioural responses, defined as behavioural fingerprints. Our results show how FDA on time series data can be effectively used to cluster behavioural response patterns with machine learning methods.

This first effective identification of contaminant behavioural fingerprints paves the way for a future application of this methodology to operational wastewater monitoring seeking to facilitate timely dedicated treatment of effluents to reduce negative environmental impacts.

#### 1.02.P-Mo024 Drainflow Prediction Based on Data-Driven Approach

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Prediction of drainflow is essential to capture the contaminants' transport to surface water bodies. Drainflow prediction with physically based numerical models (e.g., MACRO) requires a large number of field-individual input parameters and extensive computation. However, the limitations can be overcome by using data-driven approaches with a low number of inputs and fast response time. This study explores machine learning as the alternative to the physically based MACRO model. More specifically, our study evaluates the prediction accuracy of daily drainflow under heterogeneous climate conditions in Germany. Therefore, a typical drained soil type in Germany was selected and parameterized. For this soil type and each of the 817 spatially overlapping meteorological stations, daily drainflow was simulated with the MACRO model. The simulations were then used to train the data-driven model built by the state-of-the-art machine learning approach, the XGBoost (extreme gradient tree boosting). Results show that the daily drainflow prediction is most sensitive to predictors as the current and past 30 days precipitation and temperature, as well as their past five-day cumulative values. Those predictors give an impulse to the model indicating the soil moisture and precipitation change before drainflow occurs. Further, the prediction accuracy is highly positively correlated with the drainflow frequency. When the number of drained days exceeds 200 per year, the accuracy of whether drained or not ranges from 60% to 80%. However, if drained days are less than 100 per year, the accuracy may decline to 30%, possibly due to the limitation of the regression models fitting within no flow conditions. Our study demonstrates the capability of the XGBoost to simulate the drainflow and points out the options of further training refinement. Considering the vast increase of meteorological data availability, such a data-driven approach illustrates its potential to facilitate large-scale application of physically based models.

#### 1.03 Assessing Adverse Effects of Pollutants on Host-Associated and Free-Living Microbiomes using 'Omics

##### 1.03.P-Th001 Structural Responses of Bacterial Communities from Water, Biofilm, and Sediments to Environmental Parameters with Particular Emphasis on Antibiotic Stress at River Basin Level

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Microbial communities and especially bacterial communities are key players when it comes to biogeochemical processes sustaining ecosystems, but they are also responsible for degrading toxic chemicals and the development of resistance against antimicrobial drugs, which ultimately impacts human health. Bacterial responses to environmental conditions are diverse, ranging from diversity dissimilarities changes depending on their environmental compartment, type of environmental stress, and especially the type of pollution fingerprint they are exposed to. In the field of microbial ecotoxicology, there is not a standard approach when it comes to assessing the effect of trace organic chemicals derived from human activities, such as pesticides, pharmaceuticals, and personal care products, and industrial chemicals on bacterial communities; thus, the use of DNA- and RNA-

sequencing-based methods has the potential to shed light on determining alteration on biodiversity at the microbial level. In this study, environmental DNA (eDNA) was analysed from water, biofilm, and sediment samples collected in the River Aconcagua Basin (Central Chile) to evaluate: i) whether alpha- and beta-diversity changed depending on the type of land uses, ii) which environmental variables may better explain changes in community structure, and iii) to assess quantitatively and qualitatively at community level the consequences of antibiotic stress in a selected river basin characterised by intense agriculture, large mining, and the discharge of 10 wastewater treatment plants (WWTPs). Our results suggest no alpha-diversity differences between land uses for water and biofilms, while alpha-diversity was significantly lower in sediments from agriculture and nearby urban settlements. Samples from agriculture and nearby urban areas were similar in terms of their community differentiation, while reference areas were significantly different. This degree of differentiation was driven by the toxic stress caused by antibiotic mixtures, at least in two sites affected by nearby urban development and WWTP discharges. Finally, the potential ecological outcomes of these changes were assessed and significant changes in abundances of both sensitive species featuring relevant ecological functions and bacteria known for antibiotic resistance development were estimated in certain areas of the studied river basin.

### 1.03.P-Th002 Breast Milk Elemental Profile is Associated with Postnatal Maternal Factors

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Breast milk (BM) composition in macronutrients, microorganisms, immunoglobulins, or vitamins has been widely studied, but less attention has been paid to minerals, especially metals. Diet, but also other perinatal or maternal factors, could determine the composition in metals in BM, and also, the lactation time could have an impact on the elemental profile.

Breast milk samples were prospectively collected in a cohort of 99 mothers in follow-up in the MAMI project. The absolute quantification of BM elements was determined by inductively coupled plasma mass spectrometry (ICP-MS) at two lactation times. Breast milk microbial profiling by 16S rRNA amplicon gene sequencing in the Illumina platform was also obtained. Maternal-infant clinical records, perinatal factors including maternal BMI, mode of birth, and lactation stage (7-15 days and 1-2 months after birth), and maternal diet were considered.

Lactation time showed to determine the composition in metals: Cu, Zn, and Sb decreased with lactation time, while Pb increased significantly. In early samples, mothers who had vaginal delivery had significantly lower content of Al, while in the mature samples, the content of Cu and Zn were higher. In addition, mothers' consumption of animal protein and meat had higher Co and Cu in BM than those following a diet high in vegetable protein and fiber. Related to diet, we also found that the consumption of nuts and processed meat was positively correlated with As and Cd. Also, the metal composition was correlated with some bacterial genus in BM, including positive correlations between *Bifidobacterium* with Cu; *Enterobacteria* with As, Cd, and Sb; and negative correlations between *Lactocaseibacillus* and Fe and Cu. In contrast, the elemental composition in BM at the assessed lactation times was not related to any of the infant growth indicators.

It was concluded that diet can shape the composition in relevant metals for infant nutrition, including Co and Cu, while others such as Fe or Zn were not related to dietary patterns. Also, heavy metals such as As and Cd, which could have harmful effects, were related to some food groups. Other noncontrollable variables such as type of birth were also found to be a determinant of elemental composition, and in turn, the elemental composition was related to the BM microbiota. The findings of this preliminary study highlight the insights that assessing elemental composition of BM could provide to understand infant nutrition and growth.

### 1.03.T-01 Meta-Metabolomics to Uncover Short- vs Long-Term Response of Periphytic Microbiome to a Model Fungicide

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Agricultural activity leads to the release of thousands of compounds (e.g., fungicides) to aquatic ecosystems that might be harmful for exposed organisms such as periphytic microbial communities playing a key role in ecosystem functions. Although previous studies have shown adverse effect of fungicides on the structure and functions of periphytic biofilms, there is still a paucity of knowledge on short-term molecular/biochemical mechanisms involved in such long-term impairment on ecosystem function(s). This is strongly needed to support the discovery of ecosystem biomarkers. To tackle this challenge, untargeted meta-metabolomics is an approach of choice since it provides a comprehensive picture of the biochemical activity and the molecular phenotype of a community, as a result of interactions with the environment. In this context, the present study aims to highlight the link between short-term response at the molecular level (untargeted metabolomics) and long-term response at the physiological/function (photosynthesis, enzymatic activity) and structural (biomass, chlorophyll *a*) levels of periphytic biofilms to the model fungicides tebuconazole (TBZ) at environmental concentrations (5-100 µg/L). At the structural level, there was a clear increase in the amount of chlorophyll *a* at 100 µg/L of TBZ that could be associated to the potential replacement of the fungal community (impacted by the TBZ) by autotrophic organisms. At the physiological/functional level, no fluctuation of the photosynthetic yield was noted whereas some discrepancies were observed for the enzymatic activities. In particular, there was a marked difference in the β-glucosidase activity between the control and the exposed biofilms at six and 26 days. At the molecular level, faster changes of the metabolome were noted in the exposed biofilms since half of the metabolome of the exposed biofilm



had changed at three days against five days in the control. Further enrichment analysis highlighted that the pathways modulated by the TBZ changed along the exposure suggesting a shift in the metabolism of the whole communities in accordance with the changes observed in the enzymatic activities. Altogether this study highlights that tebuconazole at environmental concentration can trigger fast change of the meta-metabolome (four hours exposure) that might led to mid-long-term change of the whole community metabolism as noted for the enzymatic activities, as well as structural changes (increasing abundance of autotrophs).

### 1.03.T-02 Effects of Erythromycin on Juvenile Rainbow Trout Gut Microbiome

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Erythromycin (ERY) is a commonly used antibiotic that can be found in wastewater effluent globally. Disruption of the fish gut microbiome is an emerging area of research in aquatic toxicology because of its importance for host health. The pseudo-persistence of ERY could have impacts on the fish gut microbiome and overall health of host fishes. The overall objectives of this experiment were to: 1) Understand uptake and depuration of ERY in rainbow trout, 2) profile juvenile rainbow trout gut microbiome response to ERY, and 3) assess plasma metabolome response of juvenile rainbow trout to exposure. Juvenile rainbow trout (90-day posthatch) were exposed via diet to three concentrations (0.1, 10, and 1000 µg/g) and one solvent control in six replicate tanks (five fish/tank). The exposure period lasted for seven days followed by a seven-day depuration period with mid-gut tissue, bile, and plasma being collected for downstream analysis using 16s rDNA metabarcoding, target analyte measurement, and target/nontarget analyte measurement, respectively. The microbial community was characterized by 16s rDNA metabarcoding, and target analytes included ERY and the metabolite N-demethyl erythromycin (DM-ERY). Analytes were screened in plasma using a full MS-ddMS2 Top 10 method with both positive and negative ionization. ERY-treated fish overall showed greater Chao1 diversity at 11 days but had lower evenness at four days when compared to control. Juvenile rainbow trout were dominated by phyla *Proteobacteria* followed by *Firmicutes*. There were indications of an increase in *Proteobacteria* in the 1000 µg/g treatment group even after 7-d recovery period (14-d). Using functional analysis, several pathways associated with ERY-treatment including Bifidobacterium shunt and heme b biosynthesis indicating reduction in fermentation ability and disruption in compound synthesis, respectively. Erythromycin was detected at the greatest concentration for four days compared to seven days for 1000 µg/g treatment group in bile but opposite in plasma. N-demethyl erythromycin was detected in plasma at greater concentrations than ERY but only detected in two bile samples. Erythromycin caused perturbation of juvenile rainbow trout microbiome that continued beyond exposure and was transformed quickly in juvenile fish, leading to elevated metabolite in plasma at seven days. Future steps include finalizing the profiling of the plasma metabolome of exposed fish to better understand implications to host fitness.

### 1.03.T-03 A Combination of 'Omics Approaches to Evaluate the Effects of Metabolic Disorders of the Nonsteroidal Anti-Inflammatory Drug Diclofenac in *Mus musculus* Mice. Antagonism with Selenium

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Biological systems are exposed to a complex environment in which contaminants can interact in a synergistic/antagonistic or agonistic fashion, and for this reason, the metabolism of toxic substances should not be considered in isolation. Due to the extensive use of diclofenac (DCF) as a nonsteroidal anti-inflammatory drug, its residues have been regularly detected in surface waters around the world, causing serious health problems. On the other hand, selenium (Se) is an essential trace element well known for its detoxification properties. In this work, we evaluated the metabolic disorders of DCF in gut content, testis and brain of *Mus musculus* mice and the role of Se supplementation. *Mus musculus* mice (30 animals) were distributed into three groups: Group A (Control), mice fed rodent diet; Group B (Control+Se), fed Se-supplemented diet (0.65 mg Se/kg of chow); Group C (DCF), 20 mg DCF/Kg of chow, Group D (DCF+Se), that combines Se-supplementation and DCF exposure. Gut, testis, and brain metabolome was analysed by gas chromatography-mass spectrometry (GC-MS) and ultra-high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-QTOF). Subsequently, the data were processed by multivariate statistical analysis to identify the metabolites altered under DCF and Se exposure. Partial least squares-discriminant analysis showed significantly separated clusters among exposed groups versus control, so *Mus musculus* undergoes metabolic disorders promoted by DCF exposure. Moreover, the gut microbiota profile was determined by 16S rRNA gene sequencing following Illumina protocols and the microbially produced metabolites by untargeted metabolomics. These results confirm that exposure to DCF and supplementation with Se cause single and joint alterations in microbiota composition and in the gut, testicular, and brain metabolome. The most significantly altered metabolic pathways were glycerophospholipid metabolism in brain, biosynthesis of unsaturated fatty acids and glyoxylate and dicarboxylate metabolism in testis, and finally primary bile biosynthesis in gut content. Our results highlight the possibilities of the applied metabolomics approach as a key tool to elucidate the mechanism of toxicity of drugs and understand the metabolic response of free-living organisms to environmental issues.

### 1.03.T-04 A High-Throughput Approach to Explore the Transformation Potential of the Human Gut Microbiome for Xenobiotic Interaction and Internal Exposure

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The influence of the microbiome on human health is becoming increasingly evident. One area of concern is the transformation products (TPs) resulting from the microbial metabolization of xenobiotics in the anaerobic gastrointestinal tract. While the literature on transformation of environmental chemicals by the microbiome is growing, the effects on humans of xenobiotic-induced changes on the ecology of the microbiome and toxicity of xenobiotics altered by biotransformation on humans are still poorly understood.

In this study we explored the transformation potential of the SIHUMix (Simplified Human Gut Microbiome x) in vitro model, which can be cultivated in bioreactors. This screening tested a larger number of pesticides, which included versatile structures to facilitate possible biotransformation. After incubation we (1) assessed changes in biotic and biotic spiked samples, (2) prioritized those with significant decrease, and (3) assessed the formation of TPs.

Our findings proved the applicability of the SIHUMix in combination with nontargeted LC-MS in high-throughput screening for toxicant transformation in the microbiome for the first time. Subsequent experiments will test these findings in *in vivo* models (zebrafish, mice) and compare biotransformation of the human microbiome with previous findings on biotransformation in the environment. This is relevant for humans, but also for many other living organisms in the environment.

### 1.03.P Assessing Adverse Effects of Pollutants on Host-Associated and Free-Living Microbiomes using 'Omics

#### 1.03.P-Th003 Bioavailability of Flumequine and Diclofenac in a Mice Model Exposed to a “Chemical Cocktail.. Impact of Antibiotic-Induced Depletion of Gut Microbiota and Selenium Supplementation

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Ecosystems are exposed to a complex environment in which pollutants may interact through antagonistic or synergistic schemes, which highlights the need for global studies on the biological response of the living organisms that inhabit these ecosystems. Although there are studies on the effects of pollutants and their biological responses in living organisms, in most of them, only those produced by a substance or group belonging to a single chemical group are studied (e.g., metals, pesticides, or pharmaceuticals). Controlled exposure assays to chemical cocktails involving several families of potentially toxic chemicals can yield highly relevant information, despite their complexity in the interpretation of results, as biological systems will produce different responses due to the interaction between substances and the aforementioned synergistic or antagonistic effects.

In this project a controlled exposure experiment on mice (*Mus musculus*) to a “chemical cocktail” containing metals (As, Cd, Hg) and pharmaceuticals (diclofenac [DCF] and flumequine [FMQ]) have been realized and additionally the role of antibiotic depletion of gut microbiota and the presence of selenium as antagonist have been also evaluated.

Samples were extracted using a vortex assisted liquid-liquid extraction procedure and then measured using a chromatographic method by ultra-high performance liquid chromatography (UPLC) coupled to quadrupole time of flight mass spectrometry detection. Levels of FMQ and DCF as well for the 3-, 4-, and 5-hydroxy diclofenac metabolites were measured. Results on their bioavailability have been discussed according to exposure conditions of each test group. Additionally, a computational identification of 48 DCF and FMQ metabolites based on previously reported in literature was realized.

Selenium seemed to affect pharmaceutical absorption and DCF metabolism; however, FMQ seemed to be unaffected. Despite the presence of the metal in the cocktail, this did not seem to affect it significantly; significant differences in groups with microbiota depleted were obtained in the Se-supplemented diet group, as the depleted microbiota seemed to affect the levels of DCF considerably. The number of metabolites identified was lower when the animals were fed a metal-drug chemical cocktail.

#### 1.03.P-Th004 Exploring the Effects of “Chemical Cocktails” into Brain and Testicular Metabolomes. Intertwined Mechanisms with Gut Microbiota and Selenium

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Insights about the crosstalk between the gut microbiota and the brain through the gut-brain axis have been extensively reported as well as the potential impact of pollutants on that communication. Among pollutants, mercury (Hg) and arsenic (As) are well-known neurotoxicants, but selenium (Se) has been demonstrated to counteract this effect due to its antioxidant character and neuroprotective action. Thus, the biological effects of pollutants should not be explored in isolation and for this reason, we developed an experiment to evaluate the joint impact of a “chemical cocktail” (CC) of As, cadmium (Cd), Hg, and pharmacologically active compounds (PACs) on brain metabolism and the potential roles of Se-supplementation and gut

microbiota. Thus, 50 *Mus musculus* mice were divided in five groups: control group, CC, CC + Se, CC+microbiota depletion by antibiotics (ABX), and CC+ ABX + Se. Mice brain and testicular tissues were analysed by an analytical multiplatform based on gas chromatography-mass spectrometry (GC-MS) and ultra-high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-QTOF). Bacterial composition was determined by 16S rDNA gene sequencing using Illumina apparatus. We observed an increased mortality in groups CC and ABX-CC with the worst outcome for the ABX-CC group, but the mortality ratio returned to normal values in groups CC-Se and ABX-CC-Se. Numerous metabolites were identified in the different groups, including organic acids, fatty acids, amino acids, and glycerophospholipids in brain, as well as glycerophospholipids and sphingolipids (testis). Selenium was not capable of modulating metabolic pathways in the ABX mice model after the CC exposure. However, in spite of the key impact of the CC into the gut microbiota composition, diversity, and richness, Se modulated the abundance of several metabolites in brain and testicular tissues impacting also gut microbiota. The number of altered brain and testicular metabolites by the exposure to xenobiotics decreases after Se supplementation and they were also affected by microbiota depletion by antibiotics. Moreover, the concentration of arsenic species, Hg, and PACs varied among the different groups demonstrating the impact of Se and microbiota as well as their interactions with the xenobiotics. Our results suggest the presence of numerous intertwined mechanisms between gut microbiota, metabolites, Se, and xenobiotics that should be explored to delve into the impact of pollution real in living beings.

### 1.03.P-Th005 Impact of a Metal-Drug “Chemical Cocktail” on Mice Bile Acids Profile

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Bile acids (BAs) are endogenous steroids with a marked hepatotropic character that play important roles in the absorption and digestion of dietary fats and fat-soluble vitamins. They are also the main route of cholesterol breakdown. These endogenous metabolites are essential for monitoring general metabolism and clearly indicate alterations in metabolic pathways. Evident changes in the concentration of individual BAs and their metabolic profiles can usually be detected in response to any alteration in the organism and have therefore been considered important biochemical markers.

In this work, the main objective was to evaluate the alteration of bile acid profiles in an *in vivo* animal model (*Mus musculus*) after exposure to a “chemical cocktail” containing several environmental pollutants from different chemical groups (metals and drugs), to analyse the potential impact of their presence on the biological response of the living organisms under study. Additionally, the role of antibiotic depletion of gut microbiota and the presence of selenium as antagonist were also evaluated.

For this purpose, a controlled exposure experiment to a “chemical cocktail” containing metals (As, Cd, Hg) and drugs (diclofenac and flumequine) was carried out in mice to evaluate the effect on their bile acid profile. To this end, plasma and liver samples from exposed mice were extracted and the bile acids (cholic acid, taurocholic acid, glycolic acid, taurodeoxycholic acid, ursodeoxycholic acid, and deoxycholic acid) were analysed by liquid chromatography coupled to time-of-flight mass spectrometry detection.

The results showed significant differences in the concentration of bile acids measured in the livers of mice from the different groups. Taurocholic acid appears to be the compound with the greatest differences between the different groups analysed and therefore the compound most affected in the bile acid profile. On the other hand, the levels of deoxycholic acid, glycolic acid, and ursodeoxycholic acid decreased drastically in the exposed groups compared to the control groups.

### 1.03.P-Th006 The Influence of Florfenicol Coated Fish Feed on the Gene Expression of the Atlantic Salmon (*Salmo salar*) Gastrointestinal Tract and its Microbiome

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The use of antibiotics to treat fish diseases in aquaculture is still necessary, and dosages and treatment lengths greatly vary depending on local regulations. Although concerns about the impacts of antibiotics in aquatic organisms are reported worldwide, the potential adverse effects on commensal gut microbiota and fish health are still not fully understood. Herein, changes in the fish gastrointestinal tract (GIT) transcriptome and its microbiome due to treatment with florfenicol (FFC), a fluorinated synthetic analog of thiamphenicol and a broad-spectrum bacteriostatic antibiotic, were investigated.

Juvenile Atlantic salmon (*Salmo salar*) were treated with commercial feed coated with FFC (10, 20, and 30 mg/kg) for 18 days and afterwards were subjected to 15 days of recovery, to simulate real field scenarios. The GIT tissues were used for mRNA sequencing analysis (metatranscriptomics) to quantify gene expression in the host GIT and its microbiome. After FFC treatment, 1058 unique Enzymes Commission numbers (ECs) and 69 unique pathways were identified. No statistically significant differences were determined for ECs and pathways between treated and control groups. After recovery, 1201 unique ECs and 71 pathways were identified. A total of 9 ECs, involved in metabolic pathways and the biosynthesis of secondary metabolites, were

statistically different when comparing treatment vs. control. A dose dependent effect was observed, as measured by an increased number of differentially expressed genes (DEGs) from the low to high dose of FFC.

With regard to transcriptomics responses in the fish intestine, the top predicted canonical pathways in common between treatments were categorized as DNA replication, cell death and survival, and apoptosis, indicating potential gut inflammation. Top predicted canonical pathways after the 30 mg/kg treatment included EIF2 signaling, protein ubiquitination, LXR/RXR activation, and FXR/RXR activation, indicating potential activation of xenobiotic metabolism. After recovery, top predicted canonical pathways in common between treatments were EIF2 signaling and mTOR signaling (which are both critical for cell growth and proliferation via lipid synthesis regulation), lysine degradation II and V (lysine is an essential amino acid strongly related to fish growth), and regulation of eIF4 and p70S6K signaling. The same dose dependent effect, observed in the microbiome, was confirmed in the fish GIT.

**1.03.P-Th007 Antimicrobial Effects on the Gut Microbiome of a Leaf-Shredding Amphipod** *Alexander Feckler<sup>1</sup>, Mirco Bundschuh<sup>2</sup>, Ondrej Adamovsky<sup>3</sup>, Sven Katzenmeier<sup>4</sup>, Hana Vespalcova<sup>3</sup> and Thorsten Stoeck<sup>4</sup>*, (1)Eußerthal Ecosystem Research Station, University of Koblenz-Landau, Germany, (2)iES Landau, University of Kaiserslautern-Landau (RPTU), Germany, (3)Recetox, RECETOX, Masaryk University, Czech Republic, (4)Ecology Group, Technical University Kaiserslautern, Germany

Host-associated microbiomes support, for instance, nutrient uptake and their processing. Disturbances in such microbiomes can cause severe diseases in humans and mammals, whereas the consequences for the physiology of (aquatic) invertebrates are largely unknown. However, literature points to changes in invertebrates' gut microbiomes as a consequence of direct wastewater and antibiotic exposure, which are likely linked to physiological implications. At the same time, the impact of antimicrobials on the gut microbiome via the nutritional quality of their hosts' diet and dietary exposure to chemicals is mostly ignored. Here, we assessed the bacterial microbiome of a leaf-shredding amphipod (*Gammarus fossarum* Koch) via metabarcoding after exposure toward the antibiotic ciprofloxacin at 500 µg/L, the fungicide azoxystrobin at 15 µg/L, and their mixture through different pathways, that is water, food, and a combination of both (n = 10). We observed significant effects of ciprofloxacin, azoxystrobin, and the mixture of both on the bacterial composition in the gut microbiome ( $p \leq 0.028$ ), while only for ciprofloxacin and the antimicrobials' mixture a pathway-dependency for the observed effects were detected ( $p \leq 0.012$ ). In the latter case, the strongest effects were observed for the water exposure and the combination of both pathways. This suggests that the antibiotic was the driving force for the bacterial shifts caused by the antimicrobials' mixture. Various bacterial families were either severely suffering or benefitting from the antimicrobials' presence, while the identity of affected bacterial families was substance-specific. A benefit for bacteria in this environment points to a reduced pressure for competition, leading to a reproductive advantage. Given that knowledge on structural changes cannot directly be linked to physiological changes in the microbiomes' host, further analyses are currently ongoing. These analyses are intended to predict shifts in the bacterial metabolome and produce microbial community-wide metabolomic potential scores for a more mechanistic understanding of the physiological responses of antimicrobially exposed *G. fossarum* reported in earlier publications.

### **1.03.P-Th008 Consequences of the Fragrance Amyl Salicylate on the Mediterranean Mussel *Mytilus galloprovincialis* and on Host-Microbiota Interactions**

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Amyl salicylate (AS) is a fragrance that due to its massive use may be spread into the environment, threatening the ecosystems including wild and farmed organisms. In this regard, this substance was recently detected in water of the Venice Lagoon, becoming a hazard for animals, including mollusk bivalves such as the Mediterranean mussel (*Mytilus galloprovincialis*) that have an important economic and ecological role in this area. Despite high levels of AS occurring in water of the Lagoon of Venice, no studies investigated the possible consequences of AS exposures on species inhabiting this ecosystem to date. Thus, the present research used a multidisciplinary approach to investigate the potential effects of the amyl salicylate on Mediterranean mussels. Bioaccumulation capacity, cellular, biochemical, and molecular analyses (RNA-seq and microbiota characterization) were evaluated in mussels treated for seven and 14 days with low and high AS Venice Lagoon environmental concentrations (0.1 and 0.5 µg/L, respectively). The chemical study did not show a great bioaccumulation capability of AS, whilst the highest concentration of the tested compound led to the disruption of several key cellular and molecular processes after the long-lasting exposures. Among them, potential immunotoxicity and changes in transcriptional regulation of pathways involved in energy metabolism, stress response, apoptosis, and cell death regulations have been observed. In contrast, exposure to the low AS concentration demonstrated weak transcriptional changes and transient increased representation of opportunistic pathogens, such as the *Arcobacter* genus and *Vibrio aestuarianus* species. In conclusion, this research provides the first results of a pilot study on the effects of AS on one of the most widely farmed marine mollusk species in the Venice Lagoon and worldwide.

### **1.03.P-Th009 Impact of Contaminants of Emerging Concern on Groundwater Microbial Communities**

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Rapid urban growth and climate change are negatively impacting water resources. Additionally, water shortages are encouraging

research into potential alternative freshwater resources such as urban aquifers. As urban aquifers contain a wide range of pollutants such as pharmaceuticals (PhACs), the question is whether these urban groundwaters (UGWs) can be used as safe drinking water. Current knowledge about the microbial communities (MCs) that occur in UGWs is scarce. In addition, microbes can affect the normal attenuation processes of these contaminants or lead to opportunistic stress-resistant microbial strains. In this study, the MCs of the aquifer were analyzed to determine the microbial abundances and composition. The Barcelona city area was chosen as a pilot area due to the deterioration of the quality of the aquifers over the past years and the proven presence of numerous organic and inorganic contaminants. In this regard, river and several UGW samples were collected in March and October 2021 from the shallow aquifer of the Besòs Delta River, the main contamination source of which is a polluted river that receives discharges from wastewater treatment plants. For the analysis of PhACs, solid-phase extraction and high-pressure liquid chromatography coupled with high-resolution mass spectrometric methodology (HPLC-HRMS) were performed. A subset of samples was analyzed for the composition of the microbial communities using a 16S-amplicon sequencing approach. Preliminary results showed that approximately 40 PhACs such as anticonvulsants, antihypertensives, antibiotics, and antivirals were detected in river samples. Of these, about half were present in UGW samples. Furthermore, the sampling points located near the river compared with the farthest points showed the highest concentrations due to the majority of substances, suggesting a natural attenuation phenomenon of drugs susceptible to adsorption or redox processes. Relationships between the number and concentration of detected pollutants and microbial characteristics (diversity, abundances, and composition) were also investigated. Functionalities including key biogeochemical processes such as denitrification and ammonia oxidation within MCs were estimated by means of PICRUST2 and compared to environmental parameters. This study provides insights into the impacts of organic contaminants on the structure and function of UGW MCs and the potential role of microorganisms modulating the fate of these pollutants.

### **1.03.P-Th010 Metabarcoding of Multiple Microbial and Metazoan Taxa in Grossly Contaminated Estuaries Reveals their Differential Sensitivity to Metal Toxicity**

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Environmental monitoring in the marine environment has normally been carried out using morphological identification of macrofaunal or meiofaunal communities. These methods are time-consuming and require considerable taxonomic expertise. We have carried out DNA metabarcoding using multiple PCR primer pairs to quantify community composition of bacteria, archaea, metazoa, fungi, and diverse eukaryote microbes along a well-studied metal pollution gradient in Southwest England. The approach allows us to quantify essentially the whole range of organisms present, including neglected and poorly characterised taxa, and how this changes along a gradient from clean to severe contamination. Metal pollution has impacts on most taxonomic groups, but there are substantial differences in their sensitivity and the extent to which the influence of pollution is masked by the effects of other environmental variables.

### **1.03.P-Th011 Not Just Plastic: Understanding the Complex Micro-Environment of Wastewater Treatment Plant Microplastics and their Role in Shaping the Plastisphere**

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Wastewater treatment plants (WWTPs) are a rich source of microbes, microplastics (MPs), and organic and inorganic chemicals. Plastics provide a physical surface for microbes to settle and benthic communities to develop. Chemicals are also a key component of the plastisphere, present in both the plastic substrate and associated with the biofilm. These include both the chemical additives inherent in the plastics, and extrinsic chemicals from other sources which in the WWTP environment include such things as pharmaceuticals, biocides, and industrial chemicals. Extrinsic chemicals can become associated with the plastisphere through adsorption to the plastics and the associated biofilms. Plastics' surface properties, polymer type, and associated chemicals may all influence plastisphere community composition and consequently the risk posed to the environment when discharged from the WWTP.

Of specific concern are the pathogenic or antibiotic-resistant bacteria (ARB) which have previously been identified within the plastisphere, where it is proposed that they use plastic as a raft for biofilm formation and survival. With large quantities of MPs exiting WWTPs through either liquid effluent or biosolids the identification and characterisation of these microbes is critical.

To date, no studies have characterised WWTP plastisphere communities in New Zealand. This study aims to determine the potential impacts plastics and associated chemicals have on the microbial communities present in the WWTP system, whether certain pathogenic species survive, and what effects these communities may also have on the plastics. Next-generation sequencing was applied to identify changes in microbial biofilm community compositions on five common plastics of either virgin or artificially UV-aged condition. Biofilm was sampled from linear low-density polyethylene (LLDPE), nylon-6, polyethylene terephthalate, polylactic acid, and oxo-LLDPE paddles and glass slides after two and six weeks, and six and 12 months of constant immersion in a WWTP oxidation pond. Trace elements in both the biofilm and plastics were analysed using inductively coupled plasma-mass spectrometry (ICP-MS). A more comprehensive understanding of the potential role of wastewater plastispheres, including survival and enrichment of pathogenic microbes or ARBs and the interactions with inorganic contaminants, will highlight the need for more control strategies to prevent the release of two important classes of contaminants, microbes and trace elements, into the environment in the future.

### 1.03.V Assessing Adverse Effects of Pollutants on Host-Associated and Free-Living Microbiomes using 'Omics

#### 1.03.V-01 Insights into the Functional and Structural Alterations of a Simplified Human Intestinal Microbiota Model Caused by Pesticides Using Proteomics and Metabolomics

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The human gut microbiota is a complex ecosystem involved in key functions essential for the host. These include protection against pathogens, immune-system modulation, fermentation of nondigestible dietary fibers, and biotransformation of xenobiotics. The symbiotic intestinal microbial community is sensitive to external disturbances, caused by the interaction of the host with its environment, increasing the risk of dysbiosis and, thus, affecting the health of the host. Nevertheless, regardless of their known importance and potential ability to metabolize xenobiotics and their secondary products, microbiota models are not part of risk assessment assays, and information on the effects of environmental contaminants on the composition and function of the human intestinal microbiome is still sparse. In this study, we investigate the response of a simplified gut microbiota *in vitro* model exposed to 54 pesticides in environmentally relevant concentrations for 24 h. To examine the effect on the structure and function of the Simplified Human Intestinal Microbiota (SIHUMIX), the profiling of the taxonomic composition using metaproteomics, and the detection of short-chain fatty acids (SCFAs) through metabolomics was performed. Nineteen of 54 pesticides caused a reduction in at least one of the nine most common SCFAs detected. Ethoxyquin, flufenacet, fluoroxypyr, foramsulfuron, primicarb, prochloraz, and propamocarb significantly reduced the concentration of the main SCFAs: acetate, lactate, propionate, and butyrate. On the taxonomic level, out of the 54 pesticides, 20 produced a significant change in at least one of the SIHUMIX strains. While half of the positive hits caused a change in only one of the bacterial strains, the other half caused the simultaneous reduction of multiple gram-positive species such as *Clostridium ramosum*, *Bifidobacterium longum*, *Lactobacillus plantarum*, and *Clostridium butyricum*. This study presents a high-throughput *in vitro* approach, where multiple pesticides were screened in parallel against a stable microbiota model, and significant effects on the community's structural integrity and energy metabolism were observed. We anticipate our assay to be part of the efforts to achieve a better understanding of microbiota-xenobiotic interactions, leading to better decision-making regarding pesticide regulation in the interest of improving human health.

### 1.04 Daphnia - The Good, the Bad, and the New?

#### 1.04.T-01 Can *Daphnia magna* be used as a Surrogate Species to Predict Environmental and Human Health Defects of Pollutants

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The aquatic crustacean *Daphnia magna* is an established ecotoxicological and ecological model that is routinely used to assess the acute and chronic toxicity of new and existing chemical pollutants. Quite recently it was found that many molecular drug targets in humans were also present in the *D. magna* genome. Accordingly in USA *D. magna* has been proposed as a biomedical model. This is in line with the need to reduce animal experimentation and the increasing use of New Approach Methodologists and Adverse Outcome Pathways (AOPs) in both environmental and human risk assessment of chemical products. We present results obtained for the last 10 years by our team where we have successfully developed two *D. magna* toxicological models: the *D. magna* serotonergic neurological model sensitive to selective serotonin reuptake inhibitors (SSRIs) and the obesogenic-like model able to predict and assess the molecular mode of action and their adverse outcomes. As in humans selective serotonin reuptake inhibitors (SSRIs) increased brain serotonin levels in the *D. magna* brain and decreased the responses to stress. Obesogenic compounds enhanced the accumulation of storage lipids. Just recently we have been developing a *Daphnia* model for assessing pollutant effects on Developmental Origins of Neurobehavioral Deficits and Diseases (DONDaD) that has established a link between short-term exposure to environmental pollutants during specific developmental windows and different neurological disorders. A *D. magna* brooding female contains at least three types of developmental eggs: developing embryos in the brood pouch, and provisioned and unprovisioned eggs in the ovaries. This means that by exposing an adult female during a reproductive cycle that lasts 3-4 days at 20°C, then transferring to clean medium and collecting the first three consecutive clutches, it would be possible to assess delayed pollution mediated effects in juvenile stages upon exposure to key developmental stages. We tested DONDaD using environmental low concentrations of two SSRIS (fluoxetine and sertraline) and the obesogenic pesticide tributyltin across different experiments. Behavioural effects measured in 3-4 day-old juveniles evidenced clear stage-specific different responses among exposed juveniles and developing stages. Ongoing studies of the transcriptomic and metabolomic pathways involved in the observed developmental stage specific effects will help to elucidate the molecular mechanisms.

#### 1.04.T-02 *Daphnia* as Model System to Address Questions at the Interface of Evolutionary Ecotoxicology and Multiple-Stressor Research

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While the water flea *Daphnia* has been widely appreciated as an ideal model organism in evolutionary ecotoxicology, and increasingly in multiple-stressor research, we here show its high potential to also address outstanding questions at the interface of both fields. Despite the increasing interest in evolutionary ecotoxicology and in interactions between pollutants and natural

stressors such as warming, little is known about the genotype-dependency of these interactions within a single population. As a result, we cannot evaluate the potential of stressor interactions to evolve in a single population. Moreover, we have limited abilities to predict the interaction type (additive, antagonistic, and synergistic) between pollutants and another stressor, as it is unknown whether this can be predicted based on the sensitivities to each stressor in isolation. Capitalizing on the presence of parthenogenetic reproduction, hence the possibility to work with clones, we here demonstrate how *Daphnia* can be an ideal model organism to address such evolutionary questions at the interface of global change biology and ecotoxicology. As an example study we focused on the interaction pattern between heat extremes and pesticide exposure. We studied genotype-dependent effects of sequential exposure to a heat spike and a pesticide (esfenvalerate) in a set of six clones from the same natural population of *D. magna*. A key finding was that these clones strongly differed in interaction type whereby we identified antagonistic, additive, and synergistic interactions for two life history traits (survival and time to maturation) for the same multiple-stressor combination. These genotype-dependent interaction patterns may fuel the evolution of stressor interaction patterns and also be the underlying reason for the observed inconsistencies among studies for the same multiple-stressor combination, which also challenges current ecological risk assessment for pollutants based on single genotypes. Furthermore, by using the tolerances of the six clones to the heat spike and to the pesticide, we could show that the interaction type between the heat spike and esfenvalerate could be predicted based on the tolerance to the pesticide. Specifically, more pesticide-tolerant genotypes showed a stronger synergism. This case study therefore illustrates the potential of *Daphnia* as a model to advance our insights in outstanding topics that integrate evolutionary ecotoxicology and multiple-stressor research.

#### **1.04.T-03 Image-Based High-Content Screening of Mitochondrial Membrane Potential in *Daphnia magna***

**Cedric Abele, Amira Perez, Paula Pierozan, Magnus Breitholtz and Oskar Karlsson, Environmental Science, Stockholm University, Sweden**

The high complexity of interactions between biota and anthropogenic chemicals requires methods that are able to rapidly provide information about sublethal effects and toxicological mechanisms. The aim of this study is to optimize a high-content imaging workflow using molecular staining to assess the (eco)toxicity of chemicals in the aquatic test organism *Daphnia magna*.

JC-1 is a lipophilic cation that can permeate both the cell and mitochondrial membrane. JC-1 forms red-orange light emitting J-aggregates when the mitochondrial membrane potential is high, and stays in its green-light emitting monomeric form when the membrane potential is low. The red-to-green signal ratio is therefore an indicator of exposure for chemicals interfering with the membrane potential of mitochondria. To establish suitable protocols for JC-1 whole animal staining in *D. magna* juveniles we used the model compound 2,4-dinitrophenol (2,4-DNP), which lowers the mitochondrial membrane potential by uncoupling the oxidative phosphorylation.

We first tested different concentrations of the JC-1 staining solution and time. In the next step, we exposed juveniles to a concentration series of 2,4-DNP. Five individuals were sampled at several time points within a period of 24 h and stained with JC-1. The red and green fluorescence intensity were measured in 384 - multiwell plates. Images were acquired in an automatic multipoint confocal fluorescence microscope. An additional aim of the study is to connect the image acquisition to an automated image analysis workflow, which enables quantification of the fluorescence intensities. Based on the intensities we want to determine concentration-response relationships and compare them to the *Daphnia* sp., acute Immobilisation Test OECD Test 202 for testing of chemicals.

The study revealed JC-1 J-aggregate formation in the cells of *D. magna*, indicating that the stain did distribute to mitochondria. The results also demonstrated a concentration-response relationship between the 2,4-DNP concentration and JC-1 signal. Importantly, we were able to see effects much earlier, both in time and concentration, compared to OECD Test 202.

In summary, we demonstrated that molecular staining of whole organisms and image-based high-content screening can provide valuable toxicological data in a higher throughput manner in *D. magna*. This approach can contribute to the understanding of a chemical's mode of action, which will improve the effect assessment of chemicals.

#### **1.04.T-04 Insights into Microplastic Particles Distorting Chemical Communication**

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The abundance of micro- and nanoplastic (MNP) particles is reported in all parts of the earth, in all bodies of water, all soils, and in organisms. Several studies indicate effects of MNPs on organism and ecosystem level, but the modes of actions are not well understood. As most plastics are chemically inert, the particle effect itself is discussed not to be the sole reason for affecting organisms. One mechanism discussed frequently is the ability of microplastic particles to adsorb chemicals from the surrounding medium. In the present study, we looked at the capacity of MNPs to distort chemical communication (infodisruption) in *Daphnia magna* by removing some semiochemicals from the contaminated medium. If infochemicals are absorbed by the plastic particles and are thus removed from the system, a following subtle change in infochemical concentration will alter or distort the message. For example, changes in neurotransmitter levels will impact cognitive and perceptive capabilities, changes in the availability of pheromones and predator cues will change an individual's reproductive success and survival probability, and a small change in hormonal titres can result in an up- or downregulation of the biosynthesis of specific proteins.

We have tested in a series of experiments whether a conserved and widespread hormone (methyl farnesoate) attaches to various plastic particles. Methyl farnesoate is a hormone that induces the production of males when conditions in the environment become

unfavourable in *D. magna*. We then conducted bioassays with *D. magna* to test whether the reduction in hormone concentration due to adsorption to microplastic particles is large enough to impact the daphnids' development. Our results provide insight into how infodisruption by MNPs can alter the life history of animals and severely impact ecosystems in the process.

#### **1.04.T-05 Assessment of the Impact of Single and Mixtures Stressors on *Daphnia magna*: Using Enzyme Markers and Metabolomics as Endpoints of Physiology**

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The continuous global increase in population and consumption of resources due to human activities has a significant impact on the environment. Therefore, assessment of environmental exposure to toxic chemicals as well as their impact on biological systems is of significant importance. Freshwater systems are currently under threat and monitored. However, current methods for pollution assessment can neither provide mechanistic insight nor predict adverse effects from complex pollution. Using daphniids as a bioindicator, we assessed the impact in acute exposures of eight individual chemicals and specifically two metals, four pharmaceuticals, a pesticide and a stimulant, and their composite mixture combining phenotypic, biochemical, and metabolic markers of physiology. Results from individual chemicals showed distinct biochemical responses for key enzyme activities such as phosphatases, lipase, peptidase,  $\beta$ -galactosidase, and glutathione-S-transferase. Following, a more realistic mixture scenario was assessed with the aforementioned enzyme markers, feeding rate, and a metabolomic analysis. A clear dose-dependent effect for the composite mixture was validated with enzyme markers of physiology, and the metabolomic analysis verified the effects observed, thus providing sensitive metrics in metabolite perturbations. Additionally, exposure to the composite mixture resulted in a "shut down" of feeding. Our study highlights that sensitive enzyme markers can be used in advance on the design of metabolic and holistic assays to guide the selection of chemicals and the trajectory of the study, while providing mechanistic insight. In the future this could prove to become a useful tool for understanding and predicting freshwater pollution.

#### **1.04.P *Daphnia* — The Good, the Bad, and the New?**

##### **1.04.P-Mo025 Ecotoxicological Effects of Copper at Two Different Temperatures on — The Impacts of Climate Changes**

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In the last decades, climatic changes have been a great concern among the scientific and political communities. Nowadays, climatic changes effects have been reported associated to extreme climatic events like the rise in temperature, floods, and droughts with impacts on several activities (e.g., agriculture) and in the ecosystems. The exponential increase in world population and growth in food consumption are placing unprecedented demands on agriculture and natural resources. Hence, more research is needed, not only for a more diverse array of pesticides, but also for commercial formulations, applied in the field.

Copper belongs to the group of transitional essential metals, of vital importance for every organism at low concentrations, becoming toxic at high amounts, being considered the third most toxic metal after cadmium and mercury.

This work aimed to determine the impact of copper in a primary consumer (*Daphnia magna*) at two distinct temperatures (20°C and 25°C) and how temperature impacts those effects. Immobilization and reproduction bioassays following the guidelines for the testing of chemicals with some adaptations (reproduction) were performed. Our results show that an increase in metabolic rate induces early maturity with the 1<sup>st</sup> brood emerging at 6<sup>th</sup> day and the appearance of the 7<sup>th</sup> and 8<sup>th</sup> broods at 25°C. Furthermore, with increased exposure treatment tested induce a desynchronization in the release of the broods with the highest concentration showing a statistically significant impact on the number of broods at the highest temperature. At 25°C mortality rate was concentration dependent reaching 100% in the highest treatment. Two-way ANOVA and ANCOVA with the use of the Tukey test indicate statistically significant differences caused by the interaction of temperature and concentration and temperature ( $P < 0.05$ ) in most parameters analysed. This study highlights the importance of temperature on contamination scenarios and in the population of freshwater systems.

##### **1.04.P-Mo026 Are Microplastics at Environmentally Relevant Concentration Toxic: Evidence from *Daphnia magna* under Global Warming**

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Currently, one prominent problem in ecotoxicological studies on microplastics is that most exposure concentrations are far greater than the actual concentrations of microplastics in the environment, and microplastics are sometimes considered not harmful at environmentally relevant concentrations. Yet, such studies were conducted under standard thermal conditions and thereby ignored the impacts of higher mean temperatures (MT), and especially daily temperature fluctuations (DTF) under global warming. On the other hand, we know next to nothing about how microplastics affect the adaptation of organisms to global warming. In this study, *Daphnia magna* was exposed to an environmentally relevant concentration of polystyrene microplastics (5  $\mu\text{g L}^{-1}$ ) under six thermal conditions (MT: 20 °C, 24 °C; DTF: 0 °C, 5 °C, 10 °C) over two generations to investigate the interactive effects of



nanoplastics and global warming. Results showed that nanoplastics had no effects on *Daphnia* at standard thermal conditions (constant 20 °C). Yet, nanoplastics increased the fecundity, heat tolerance, amount of energy storage, net energy budget, and cytochrome P450 activity, and decreased the energy consumption when tested under an increased MT or DTF, indicating a hormesis effect by nanoplastics under global warming. Notably, the increased heat tolerance indicated nanoplastics could induce adaptation of *Daphnia* to warming, which was due to the reduced energy consumption and/or increased energy availability. Overall, the present study provided novel insights for the toxicity studies of nanoplastics and highlighted the importance of including DTF and thermal adaptation in the realistic ecological risk assessment of nanoplastics under global warming.

#### **1.04.P-Mo027 Unexpected Interactive Effects of Nitrate and Heatwave Exposure on the Survival, Growth, and Reproduction of *Daphnia magna***

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Due to eutrophication, freshwater ecosystems are increasingly exposed to anthropogenic stressors such as nitrate. Additionally, climate change causes more intensive and frequent heatwaves, which have a tremendous impact on all trophic levels of the ecosystem. Any change in the lower phytoplankton trophic level may also impact the higher trophic levels such as to the *Daphnia magna*. Individual effects of heatwaves, high nitrate concentrations, and changing food quality have been studied on *Daphnia magna*, but less is known about their interactive effects. In this study a 3x3x2 factorial design was used where 48-hour-old *D. magna* were exposed to combinations of ecologically relevant nitrate concentrations (0, 50, or 200 mg/L) and different heatwave scenarios (no, short, or long). They were either fed with a control diet or an experimental diet of microalgae that were exposed to the same conditions as the *Daphnia*. Throughout the 45-day-long experiment, the interactive effects of nitrate, temperature, and feed on mortality, maturation, number of offspring, and body size were evaluated. Although it was suspected that exposing *D. magna* to a long heatwave scenario in combination with a high nitrate concentration would result in an overall low performance, this was not the case. Results show that *Daphnia* cultured in the absence of nitrate had a longer maturation time, higher mortality, smaller body size, and a lower number of offspring, regardless of the temperature or the type of feed. The restricted continuous proliferation of microalgae at the nitrate-limited condition (0 mg/L) reduced the food availability causing this higher impact on *D. magna* life history traits. Heatwaves shortened the lifespan of the *Daphnia*. Daphnids cultured in high (200 mg/L) nitrate with control feed performed better than with experimental feed, which indicates that at the high nitrate condition, the experimental phytoplankton was either unable to meet the energy requirements or it introduced extra stress to the *Daphnia*. Interestingly, quantity and quality of the feed had a higher impact on daphnids than nitrate concentration or temperature. Nevertheless, nitrate and temperature cannot be dismissed as stressors for *D. magna* as they clearly impact the algae that *D. magna* feeds on.

#### **1.04.P-Mo028 Resurrected *Daphnia* as Indicators of Microevolutionary Adaptive Mechanisms to Multiple Stressors**

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The resilience of an organism is characterized by good adaptation and the resulting robustness to constantly changing environmental conditions. Due to increasing anthropogenic influences such as intensification of agriculture, population growth, advancing global industrialization, and the climate change associated with it with increasing evidence, conditions in different ecosystems are changing rapidly, making quick and targeted adaptation difficult for many organisms. However, ecotoxicology is generally concerned with the acute and chronic effects of contaminants on aquatic organisms and the environment over a short period of time, which would not consider genetic adaptations in exposed populations. Thus, long-term studies are critical to understanding ecological and evolutionary processes in nature and in assessing how species respond to and persist during environmental change. The emerging field of the evolutionary toxicology examines effects of pollutants on organisms both at the organismic and the molecular level. When combined with resurrection ecology approaches, evolutionary toxicology provides a novel opportunity to examine evolutionary adaption at the genetic level to the deteriorating environment conditions over a long period of years to centuries without the traditional limitations of long-term or multigenerational studies. Resurrection ecology utilizes dormant eggs, for example, of the keystone species *Daphnia*, retrieved from dated lake sediment cores that are hatched from over years to centuries ago.

The aim of this research is to examine the effects of climate change and chemical exposure on the model keystone organism *Daphnia magna* using resurrection ecology approaches. The chemical test substance is the polycyclic aromatic hydrocarbon (PAH) phenanthrene. Phenanthrene is a common PAH, often found in the environment due to incomplete combustion of fossil fuels and offers key insights into historical exposures through chemical profiles archived in lake sediment profiles. Chronic (21-d reproduction test, OECD 211) and acute (Immobilisation test, OECD 202) effects on resurrected ancient and modern *D. magna* clones from four different lake phases of Ring Sø (Brædstrup, Denmark) will be investigated using standardized bioassays to assess apical endpoints. Furthermore, RNA sequencing, a high-throughput next generation sequencing method, will be used to analyse stress-related gene expression patterns, encoded within the RNA of *Daphnia*.

#### **1.04.P-Mo029 Cadmium Chloride Toxicity across 20 *Daphnia magna* Clones**

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*Daphnia magna* is recommended by the OECD as a test species for acute ecotoxicity testing. Although EC50 responses to

toxicants can vary across different clonal lines, test guidelines do not specify a *D. magna* strain/genotype. We used *D. magna* lines resurrected from the sedimentary archive of a lake with documented changes in chemical pollution over time and exposed 20 of these lines to cadmium chloride. The observed EC50 varied significantly among lines up to two orders of magnitude. The genetic variation among clonal lines, as well as historical environmental stress, may contribute to significant variation in reported EC50 values for chemicals. This study shows that extrapolating EC50 levels from a single strain may lead to under- or overestimation of toxicity. It potentially points to the limited power of EC50 estimates for environmental risk assessment.

#### **1.04.P-Mo030 Effect of Historic Pesticide Exposure Upon Response to DDT in *Daphnia***

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*Daphnia* are a keystone species in freshwater communities, and their response to environmental stress can be used to infer wider community response in small-scale laboratory settings. Changes in their reproduction and transcription are often used to understand the effect of environmental toxicants such as pesticides. The insecticide DDT was widely used until the second half of the 20th century when it was banned in many areas of the world, in part due to its nature as a persistent organic pollutant and its possible adverse effects on human health. However, it is still used today in parts of Africa and East Asia for effective control of disease-bearing insects.

In this study, we utilise *Daphnia* resurrected from a sedimentary archive with known historical presence of DDT to assess how historical exposure to chemical stress may affect naïve and experienced *Daphnia* genotypes to DDT exposure. Using liquid chromatography-mass spectrometry (LC-MS), we measured DDT in the sediment of the lake from which the *Daphnia* originated.

We study fitness-linked life history traits and biomolecular responses of *Daphnia* to environmentally relevant concentrations of DDT, and identify evolutionary mechanisms underpinning response to recurrent and novel stress.

The potential impact of novel chemical stress on the keystone grazer *Daphnia* has important implications for aquatic food webs, given its central role in lentic freshwater environments worldwide.

#### **1.04.P-Mo031 Chronic Exposure of *Daphnia magna* to Insecticides: Unconventional Effect on Reproduction and Sensitivity of Behaviour as Biomarker**

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Aquatic organisms are chronically exposed to a multitude of pollutants over several generations. Chronic exposures at low concentrations can induce physiological changes and thus have direct adverse effects on populations. Nowadays, classical endpoints measured during chronic exposure focus on reproductive endpoints. However, other parameters may be impacted by long-term exposure and could be interesting to study regarding the ecological relevance of the test results. The behaviour is one of those parameters. Indeed, change in the behaviour can affect the population survival. For example, the organisms may no more be able to escape predation or to feed. In my study, I aimed in highlighting the physiological changes in *Daphnia magna* (reproduction, mortality, and size) through a parental exposure of 21 days to concentrations of 0.1 to 300ng/L to the insecticide diazinon. I also evaluated the effect of the pollutant on the swimming behaviour of organisms. Indeed, diazinon, as an acetylcholinesterase inhibitor, is expected to affect the behaviour of the daphnids. The first results show a significant decrease of the cumulative reproduction over 21 days from the lowest concentration at 0.1ng/L with an increase of this phenomenon up to 33ng/L before decreasing to the normal level from 100 to 300ng/L. On the other hand, the size of the organisms after 21 days does not show any significant difference. The effects on swimming behaviour are currently being processed.

#### **1.04.P-Mo032 Differential Susceptibility to Arsenic in Glutathione S-Transferase Omega 2 (GST-O2)-Targeted Freshwater Water Flea *Daphnia Magna* Mutants**

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To examine the role of glutathione S-transferase omega class (*GST-O2*) genes in the biotransformation and detoxification in *Daphnia magna*, various responses such as *in vivo* endpoints, arsenic speciation, enzymatic activities, and gene expression pathways related to arsenic metabolism were investigated in wild-type (WT) and *GST-O2*-targeted mutant (MT) fleas produced by CRISPR/Cas9. Sensitivity to arsenic in MT fleas was higher than in WT fleas. Also, the reduction rate of arsenate ( $\text{As}^{\text{V}}$ ) to arsenite ( $\text{As}^{\text{III}}$ ) in the MT group was significantly lower and led to accumulation of higher arsenic concentrations, resulting in decreased protection against arsenic toxicity. Relative mRNA expression of other *GST* genes in the *GST-O2*-targeted MT group generally increased but the enzymatic activity of GST decreased compared with the WT group. Oxidative stress on arsenic exposure was more strongly induced in the MT group compared with the WT group, resulting in a decrease in the ability to defend against toxicity in *GST-O2*-targeted mutant *D. magna*. Our results suggest that *GST-O2* plays an important role in arsenic biotransformation and detoxification functions in *D. magna*.

#### **1.04.P-Mo033 Toxicity of Atmospheric Particulate Matter from a Brazilian Industrial Area on *Daphnia magna***

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Mining, iron, and steel industries are a continuous source of air pollution due to the amount of atmospheric particulate matter (PM) they release. This PM is a complex mixture formed by metallic nanoparticles and metals, which can reach aquatic ecosystems and may have significant ecological consequences. The aim of this study is to evaluate the toxicity of the PM, collected in a Brazilian region (State of Espírito Santo, Southeast Brazil) influenced by the steel and iron mining industry, and to know the possible impact it may have on aquatic organisms. For this purpose, the crustacean *Daphnia magna* was exposed to different environmentally relevant concentrations (0.01, 0.1, 1, 5, 10 g/L). The endpoints studied were: avoidance throughout 24 h in a nonforced exposure system, reproduction (number of neonates per female after 21 days of exposure), acetylcholinesterase activity (AChE) after 48 h, and finally, feeding rates in a short-time exposure (48 h) and in a long-time exposure (21 day + 48 h). As results, there was a negative effect of this material on the organisms, with the exception of reproduction, which was increased as from 1 g/L and the first brood occurred earlier as from 5 g/L. The avoidance was concentration-dependent and represented 88% and 100% at the two highest concentrations. The AChE activity was significantly inhibited in 5 and 10 g/L. The postexposure feeding rates were lower in long-term exposure at the highest concentration. According to our results, the concentrations with statistically significant differences were 5 and 10 g/L. In addition, in order to explain this material's toxicity, a chemical analysis was performed to characterize the metals present in this compound, but no direct relationship was observed. This study highlights the need to understand the toxic effects generated by metal mixture present in MP coming from anthropogenic activities on aquatic organisms, to perform adequate safety regulations, because of their toxicity, persistence in the environment, and potential bioaccumulation in living organisms.

#### 1.04.P-Mo034 Establishing the Effects of Aquatic Pharma-Pollution on Female *Daphnia magna* Strauss 1820 Biological Organization

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Contaminants may cause adverse effects at all levels of biological organization, manifested from molecule to ecosystem levels. Illuminating the cause-and-effect linkage between stressors and responses across the levels of biological organisation affords advances in environmental risk assessment. We investigate the effect of emerging contaminants detected in surface waters on the aquatic ecosystem.

To improve our understanding of anthropogenic pharmaceutical pollution within aquatic environments, we investigated aquatic pharma-pollutants within the well-characterized *Daphnia magna* model. Bioaccumulation of contaminants within the tissues of adult female *Daphnia magna* Strauss 1820 acutely exposed to environmentally relevant venlafaxine concentrations as a single compound as well as a mixture containing 11 emerging contaminants categorised as Watchlist compounds by The Water Framework Directive (metformin, gemfibrozil, sulfamethoxazole, trimethoprim, venlafaxine, carbamazepine, diclofenac, erythromycin, clarithromycin, azithromycin, and gabapentin) were quantified using liquid chromatography-mass spectrometry (LC-MS) analysis. Initial monitoring results for Watchlist chemicals identified that venlafaxine is the most predominantly observed analyte in surface waters. Therefore, this analyte has been chosen in this study of biological effects.

The chronic effects of the pharmaceutical were then established by observing transgenerational effects on organism survival, reproduction, morphology, organ function, and epigenetic alterations. Preliminary results have shown the bioaccumulation of contaminants within *Daphnia Magna* Strauss 1820 tissues. Analysis of reproduction studies on individuals chronically exposed to environmentally relevant concentrations of venlafaxine shows a decrease in offspring numbers with an increase in heart rate. The study aims to further correlate transcriptomic alterations investigated by RNAseq analysis to population effects including body length and width of first-generation females (F0) and first filial (F1) individuals.

#### 1.04.P-Mo035 Acute Toxicity, Oxidative Stress, and Apoptosis due to Short-Term Triclosan Exposure and the Multi- and Transgenerational Effects in the Freshwater Water Flea *Daphnia magna*

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In this study, the median lethal concentrations of triclosan (TCS) were determined as 184.689 and 349.511 µg/L in neonate and adult stages of the freshwater water flea *Daphnia magna*, respectively, based on the acute toxicity assessment. Furthermore, 50 and 100 µg/L TCS have induced oxidative stress and the changes of reactive oxygen species (ROS) content and antioxidant enzymatic activities in *D. magna* have been analyzed. However, several apoptosis-mediated proteins showed TCS-induced oxidative stress damage in response to 25 µg/L TCS, indicating that apoptotic proteins were the most sensitive mediators. Also, the multi- and transgenerational effects of TCS were evaluated in *D. magna* over three generations on various *in vivo* endpoints, DNA damage responses, and biochemical reactions. The transgenerational group exposed to TCS showed more negative impacts on antioxidant responses, DNA fragmentation status, and biological endpoints than the multigenerational exposure group, leading to decreased reproduction and higher ROS content. The transcriptional expression levels of glutathione *S*-transferase genes in the transgenerational exposure group were upregulated compared to those in the multigenerational group, but they were fully recovered in the F2 offspring group. Our findings provide an in-depth understanding of the adaptive effects of multigenerational TCS exposure groups.

#### 1.04.P-Mo036 Toxicity and Phototoxicity of UV Filter and Microplastics Mixtures on *Daphnia magna*

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In aquatic environment, microplastics can adsorb other pollutants. One of these pollutants is ultraviolet (UV) filters. Although some countries prohibited the use of UV filters, they are still contained in personal care products and commonly used. Therefore, this study aimed to evaluate the combined toxic and phototoxic effects of polystyrene (PS) microplastics and UV filter avobenzone on aquatic organism, *Daphnia magna*. In an acute toxicity test, *D. magna* were exposed to single and combined test solutions according to UV irradiation for 48 h. The test concentration of microplastic was 20 mg/L and avobenzone concentrations were 7.5 mg/L and 10 mg/L. To assess the phototoxicity, test solutions were UV irradiated for 6 h before *D. magna* was exposed. Significant immobilisation rates were observed in the single and combined groups, and the immobilisation rate increased in the UV irradiated groups. In the combined group, despite higher food uptake, they did not growth well. In addition, *D. magna* reserved more energy in the UV irradiated groups compared to the non-UV irradiated groups. Although *D. magna* obtains energy through food uptake, they reserve energy for survival rather than growth. The results of this study indicated that the combination of microplastics and ingredients of personal care products under UV irradiation can have adverse effects on the aquatic organism *D. magna*.

#### 1.04.P-Mo037 Molecular Consequences of Traditional and Biodegradable Microplastic Exposure in a Keystone Freshwater Crustacean

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*Daphnia* are a well-established test organism for aquatic toxicants, and to date have been widely used to determine the environmental risks of chemicals and more recently nanomaterials and microplastics (MP). With the growing interest and concern about the potential impacts that MP can have on individuals and ecosystems, the environmental sampling and toxicity studies have increased in variety and complexity, covering a range of test organisms and MP combinations (i.e., polymers, morphologies, sizes, ages/weathering, or degradation stage, etc.). Studies exploring the toxicity effect of MP in *Daphnia* have expanded from high dose and short-term (acute) exposures into increasingly realistic exposures, over longer time frames with varying doses and environmental conditions. Through these increasingly realistic exposures, we can use *Daphnia* as a model to better understand how MP may be affecting the aquatic ecosystem, and to determine what are the driving factors of these toxicity responses.

*Daphnia magna* were used as a model and exposed to a panel of different microplastic fragments. Polymers included polyethylene, polystyrene, cellulose acetate, and polylactic acid to determine the effect of traditional polymers compared to newer biodegradable (under specific defined conditions) polymers. Fragments were created in the lab from spherical beads, before sequential sieving into the correct size fraction for exposures. Following this, the fragments were characterised to determine their distribution in the test medium and an initial dose response was quantified for each polymer, before a chronic exposure at the EC20 concentration from the acute studies was undertaken. During the chronic exposure, life history data were captured including total neonates, time to first brood, and total growth over time. Following this, *Daphnia* were frozen for RNA extraction, and samples were taken to determine the epigenetic changes in the *Daphnia* relative to the unexposed control group. This enabled molecular level toxicity analysis to establish if there were any sublethal changes in the daphnids gene expression as a response to the different polymer exposures. By exposing genetically identical *Daphnia magna* (Bham 2 clonal lines) we can quantify the response to traditional and biodegradable polymers, to determine if the presence of biodegradable polymers poses less of a threat in the environment compared to traditional polymers that have been the focus of most toxicity studies to date.

#### 1.04.P-Mo038 Environmental Exposure Effects: Making a Predictive Model for Acute Toxicity to Nanomaterials due to Difference in the Environmental Conditions

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The importance of the environmental parameters in toxicity testing of nanomaterials (NMs) is being increasingly recognised due to the reactive surface area and NMs' responsiveness to their surroundings, for example, the importance of the dissociation constant values, which relate to the surface charge of the NMs, and how they are influenced by the environmental conditions such as pH during NMs' exposures or environments, or how NMs interact with natural organic matter (NOM). In addition, NOM has been shown to bind to NMs and can act as a natural stabiliser, by binding to the surface of the particles and preventing agglomeration.

*Daphnia magna* have been widely used in aquatic toxicity testing of NMs to date, and a wealth of data on the impacts of exposure conditions and toxicants is currently available which can allow ranking of toxicant hazard. However, less considered to date is the impact of environmental parameters of the exposure, for example, the role of pH, specific salts such as sulfides, or the presence of NOM, we can make predictions about how NM toxicity may differ or evolve over different exposure periods. In this study, *Daphnia* were exposed to a panel of metal and metal oxide NMs under a range of different exposure conditions including pH, NOM content, and temperature to see how acute toxicity to *Daphnia* changes. These data then feed into a predictive quantitative structure-activity (QSAR) model to determine which parameters are the main drivers for toxicity.

In addition, this study is also used as a case study for how instance maps used to visualise the key steps in an experiment and especially those steps where a transformation of the NMs' physicochemical properties might occur such as upon dispersion, upon passage through daphnid guts, etc., whilst also linking together the different protocols and data capture templates. This can be

used to highlight the role of the environmental parameters in these NM transformations and/or on organism health, and how the NMs or organisms influence or change the environmental conditions. Through this, we can ensure that the metadata needed to support the environmental variability and understanding within the predictive toxicity model is also captured, to develop this to its full potential.

#### **1.04.P-Mo039 Investigating the Effect of Pesticides on *Daphnia* Clonal Populations using a Stochastic Model**

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*Daphnia* is widely used in long-term population experiments to identify sublethal pesticide effects and assess chronic toxicity. However, population experiments are often laborious and subject to stochasticity (demographic, environmental, and genetic). Therefore, large variation among a limited number of replicates can lead to misleading results based on classical statistical analyses. Model-based analysis can support the interpretation of such experimental data to identify sublethal pesticide effects. The goal of such an analysis is to identify the most important processes that determine population dynamics and lead to stochasticity, and describe them in the model. The main challenge for this task is choosing an adequate level of model complexity. Models should be as simple as possible and as complex as necessary for an adequate description of the system.

In this study, we used a stochastic, age structured population model applied to data from long-term population experiments. Experiments consisted of three different clonal populations of *Daphnia galeata* in 1L aquaria with and without chronic pesticide treatments (Diazinon and Diuron) at sublethal concentrations. The clones were selected from three lakes in Europe, characterised by different levels of pollution. We tested different formulations of fertility and mortality and compared the inferred parameter estimates from the different treatments. We used a systematic model selection process based on a nested design of the model structure and Bayesian inference to identify mechanisms from population experiments.

Posterior analysis helped to choose an adequate model description for life-history characteristics under the specific experimental conditions: a zero-inflated negative binomial distribution for fertility and mortality without density dependence. For the Diazinon treatments, a comparison of the inferred posterior parameter distributions indicated the need for a mortality rate that increases with time, indicating cumulative chronic toxic effects of Diazinon for all the *Daphnia* populations. Furthermore, different *Daphnia* clones produced eggs in the different treatments, based on their previous adaptation to levels of toxicity. With this study, we demonstrate how we can use stochastic models to infer mechanisms from population data to help identify sublethal pesticide effects. The model-based analysis can also help to support the optimization of the experimental design of future studies.

#### **1.04.P-Mo040 Automated Evaluation of *Daphnia* Neonate Number and Size in Ecotoxicological Assays**

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The evaluation of offspring number is often one of the most frequently evaluated endpoints in conventional long-term ecotoxicological tests (e.g., the *Daphnia magna* reproduction test, OECD test guideline 211, 2012). Neonate size is also a very important endpoint that has been additionally evaluated in a variety of studies such as in multigenerational toxicity tests. The evaluation of these endpoints is very demanding in time- and sampling efforts, and requires larger numbers of test organisms compared to conventional long-term ecotoxicological tests, adding extra effort counting the offspring and evaluating neonate size. An automated counting and neonate size estimation would be mostly useful in assessing very large numbers of offspring such as in the continuation of reproductive success over multiple generations.

This study aims first to evaluate an automated device to count and estimate neonates' size in reproduction tests using *Daphnia magna* as a model organism. Secondly, upon validation, the methodology was applied in a classical reproduction test (NaCl: 0.0 gL<sup>-1</sup>, 1.0 gL<sup>-1</sup>, 2.0 gL<sup>-1</sup>, 4.0gL<sup>-1</sup>), being brood neonate amount, and neonate size automatically evaluated. Manual and automated approaches have been compared.

Results indicate high correlation ( $r^2 > 0.9$ ) comparing manual and automated counting and neonates' size estimation. Thus, the use of the automated approach indicates no deviation from the classical reproduction bioassay, but minimizing time and labor intensity.

Although being a highly useful tool in the conventional *Daphnia* reproduction test, the automated computer counting would be mostly useful in assessing the reproductive success over multiple generations, minimizing both costs and labor intensity, mostly when involving endless brood neonate counting and neonate size evaluation that have limited the use of multigenerational toxicity tests and thereby its incorporation as standard procedures.

#### **1.04.P-Mo041 Exposure to Artificial Light at Night Adversely Affects Reproductive Output in *Daphnia magna***

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Light pollution imposes novel challenges on species living in urban/suburban areas and has become an emergent issue of

environmental concern given the human health and detrimental ecological effects. Anthropogenic-induced changes to light conditions and artificial light at night (ALAN) can disrupt biological rhythms and alter biological fitness through inhibition or suppression of the hormone melatonin. This time-keeper hormone plays a pivotal role of relaying information about changes in day-length and modulates circadian behavioural responses and many physiological and reproductive processes. However, effects of light pollution and of ALAN are seldom studied in aquatic invertebrates.

Here we present preliminary data on the effects of ALAN based on a standard 21-d life table experiment with the cladoceran *Daphnia magna*, for which the circadian pattern of melatonin production is well known. Artificial light at night was simulated using white LEDs with a high content of blue light in the emission spectra and are being considered a more economical solution for urban lighting. Daphnid neonates (<12 h old) were used and in the ALAN treatments had daylight as in the control photoperiod (dark nights) but during the night period were exposed to low light intensity (1 and 10 lux) which is considered relevant for aquatic ecosystems under light pollution. These ALAN treatments were crossed with an exogenous melatonin treatment (1 µM) here used as a positive control (to counter the expected ALAN-induced reduction of internal melatonin concentrations) to further investigate the physiological role of melatonin and a mediator of light pollution effects in *Daphnia*.

Results show that exposure to ALAN adversely affected *D. magna* reproductive output with significant reductions in the number of broods, total number of offspring per female, and also the intrinsic rate of population increase (r) observed for organisms exposed to 10 lux during night periods. Significant effects of the interaction (ALAN x Melatonin) do suggest that melatonin can indeed be involved in the observed response to ALAN and call for research into its role in *D. magna* response to stress. Ongoing work is exploring effects of ALAN on other endpoints such as oxygen consumption and feeding rates to better understand how shifting light conditions can influence fitness and tolerance of *Daphnia* populations in urban aquatic ecosystems.

#### **1.04.P-Mo042 Alterations of Swimming Behaviour of *Daphnia magna* upon Acute and Chronic Exposure as a Sensitive Endpoint**

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The crustacean *Daphnia magna* is a useful model organism widely used in ecotoxicological testing with a substantial amount of toxicological data available for this organism. *D. magna* has a strong phototactic behaviour characterised by swimming toward (positive phototaxis) or away (negative phototaxis) from light and is therefore highly suitable to study behavioural responses. *Daphnia* swimming is in general dependent on body size and can also be affected by factors such as light, water temperature, presence of food, and predators. Changes in the swimming behaviour of daphnids can be early indicators of stress and later on also for immobilization.

So far, according to the OECD Guidelines 202 and 211, immobility and reproduction are the main endpoints used for the determination of toxicity. Sublethal effects can be induced by chemicals at lower levels and may require more sensitive biomarkers. As a first step alterations of swimming behaviour, phototactic behaviour, and response to kairomones should be evaluated upon exposure to pharmaceuticals in environmentally relevant concentrations.

*D. magna* is exposed to a series of substances for 48 h and 21 d in order to evaluate the classical acute and chronic effects on immobilisation and reproduction, according to the OECD Guideline tests (202 and 211). Furthermore, the behavioural endpoints of *D. magna* will be investigated during acute and chronic exposure at different time points. In addition, in the reproduction tests with chronic exposure for 21 d beside the parental daphnia (F0 generation) also the juvenile daphnia (F1 generation) are investigated, to evaluate generational effects and differences in sensitivity.

The results of the different tests will be evaluated with respect to the sensitivity of the different endpoints and time points considered (classical ECx values vs. behaviour). In addition, the effects of each test substance will be compared with respect to the sensitivity of the different behavioural endpoints. The different behavioural endpoints will be evaluated according to their applicability, to use the behavioural endpoints as a more sensitive endpoint allowing a predictability of substance effects.

#### **1.04.V *Daphnia* — The Good, the Bad, and the New?**

##### **1.04.V-01 Evaluation of Possible Vardio- and Neurotoxic Effects by Methyl, Ethyl, Butyl, or Propyl Parabens in *Daphnia magna***

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Parabens have endocrine-disrupting effects, which means they can alter the hormonal control of energy metabolism, causing obesity and metabolic health problems, as well as cardiovascular and neurological problems. However, there is presently inadequate proof of parabens' adverse health effects on aquatic species. Our goal is to evaluate if the four parabens (methyl, ethyl, butyl, and propyl parabens) have any adverse effects on *Daphnia magna*'s cardiovascular and neurological systems. Physiological tests (heart rates and thoracic limb activity) and gene transcription analyses were performed 48 h after acute exposure to *Daphnia magna* at various concentrations. The heart rates and thoracic limb activity of the exposed daphnids were inhibited in a time and dose-dependent manner. The butylparaben group had the highest heart rate inhibitions. Butylparaben once again demonstrated the strongest significant suppression of thoracic limb activity in the highest concentration group. Ethylparaben and propylparaben had

no effect on gene transcription in the cardiomyopathy group. At least one gene in the GABAergic synapse and acetylcholine receptor groups was substantially affected by all parabens. The upheaval in *Daphnia magna*'s heart rate and limb activity produced by the four examined parabens is extremely considerable, according to our findings. Unexpectedly, the cardiomyopathy group had fewer genes that were significantly impacted. Exposure to parabens does not appear to have had a significant impact on the nervous system. Nonetheless, with its high sensitivity to environmental changes *Daphnia magna* is a suitable species for evaluating cardiotoxicity and neurotoxicity of chemicals. More research is needed, and is now underway, to fully comprehend the complex mechanism by which the neurobehavioural system of *Daphnia magna* is impacted.

#### **1.04.V-02 Transgenerational Response to Novel Chemicals in *Daphnia*: Implications for Remediating Chemical Mixtures**

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Anthropogenic chemicals used in most production processes have not been comprehensively assessed for their risk and impact on humans and wildlife. Current toxicological approaches employ acute toxicity tests using unrealistic chemical concentrations that organisms rarely encounter in the environment to ascertain their safe use, ignoring the long-term pathological effect of exposure to environmentally relevant concentrations. Legacy and persistent chemicals are transported globally and usually end up in the environment as unintentional pollutants causing long-term adverse effects. They can cause the deterioration of ecosystem services whilst negatively impacting humans and wildlife. Yet the chronic impact of these chemicals is rarely assessed.

Here, we study the transgenerational impact of ecologically relevant concentrations of five chemical groups on the keystone species, *Daphnia magna*: the industrial chemical PFOS, the pharmaceuticals diclofenac and trimethoprim, the biocide atrazine, and the heavy metal arsenic. We exposed *Daphnia* genotypes resurrected from a lake with a known history of chemical pollution to these chemicals to understand how historical exposure to chemicals influences adaptive response to novel chemical stress. We measured within- and transgenerational plasticity on fitness traits after exposure of “experienced” and “naïve” genotypes. As the revived *Daphnia* originates from the same genetic pool sampled from different times in the past, we were able to quantify the long-term evolutionary impact of chemical pollution by studying genome-wide diversity and identifying functional pathways affected by historical chemical stress. Our results suggested that historical exposure to chemical stress reduced genome-wide diversity leading to lower cross-generational tolerance to novel chemical stress. Lower tolerance is underpinned by reduced gene diversity at detoxification, catabolism, and endocrine genes in experienced genotypes. We show that these genes sit within pathways that are conserved and potential chemical targets within humans and other species.

#### **1.04.V-03 Transcriptional Responses of *Daphnia magna* to Sublethal Cu and Zn Exposures**

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Metal contamination in aquatic environments is a major concern due to their persistence and has received a great deal of attention in recent years. The waterflea *Daphnia magna* is an important model species for metal toxicity studies and water quality assessment. However, most of the research has focused on physiological endpoints such as mortality, growth, and reproduction in laboratory settings and neglected toxicogenomic responses. In this study, we analysed *D. magna* transcriptomics responses to Cu (120 µg/L) and Zn (300 µg/L) exposures in an environmental water obtained from a pristine lake with the adjust water hardness of 150 mg/L CaCO<sub>3</sub>. Exposure levels for transcriptomic analysis were determined by calculating EC<sub>5</sub> values for each metal at the end of a 96-h exposure with varying metal concentrations. A total of 2688 and 3080 genes were found to be differentially expressed (DEG) between control and Cu and Zn, respectively. Of these, 1793 gene differentially expressed were common for both Cu and Zn, whereas there were 895 unique DEGs for Cu and 1287 DEGs unique for Zn. Gene ontology enrichment was carried out to identify molecular functions and biological processes affected by the metal exposures. Results revealed some of the well-known biomarkers as well as novel targets for metal toxicity screening at a genomic level. Overall, this work was carried out to gain insight into the copper and zinc regulated stress response mechanisms in *D. magna* at transcriptome level.

#### **1.04.V-04 Computer Classification Methods for Gene Selection in *Daphnia magna* Toxicogenomics**

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Zinc is an essential element that influences many cellular functions. Depending on bioavailability, zinc can cause both deficiency and toxicity. Zinc bioavailability is influenced by water hardness; therefore, water quality analysis for health-risk assessment should consider both the zinc concentration and the water hardness. However, exposure media selection for traditional toxicology tests are set to defined hardness levels and do not represent the diverse water chemistry compositions observed in nature. Moreover, these tests commonly use whole organism endpoints such as survival and reproduction that require high numbers of test animals and are labour intensive. Gene expression stands out as a promising alternative that provides insight to molecular events that can be used for risk assessment. In this work, we applied machine learning techniques to classify the zinc concentrations and water hardness from *Daphnia magna* gene expression by using quantitative PCR. A method for gene ranking was explored using techniques from game theory, namely Shapley values. A 10-fold cross-validation was used to compare ranking algorithms. The best accuracy for hardness classification was achieved by using Quadratic Discriminant Analysis (QDA) with 0.88±0.10 followed by Random Forest (RF) with 0.86±0.12 accuracy. However, QDA resulted in a significantly lower accuracy for zinc and combination classification. Neural Network (NN) and RF were the best classifiers resulting in consistent and high accuracy for all classifications tested. The machine learning algorithm was able to predict sample source correctly up to 92% accuracy. The results show that standard machine learning classifiers are capable of classifying both zinc concentrations and

the water hardness simultaneously, and that Shapley values are a versatile and useful alternative for gene ranking, providing insight into the importance of individual genes.

### **1.05.P Epigenetic Changes Modulating the Response of Organisms to Environmental Challenges: From Phenotypic Effects to Microevolution Patterns**

#### **1.05.P-Th012 Incorporating Epigenetics in Adverse Outcome Pathways: The EPIBOOST Project**

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Lately, environmental studies started appraising several 'omics, e.g., epigenomics, as remarkable tools to improve Ecological Risk Assessment (ERA) frameworks. Besides the mechanistic enlightening of toxicity that these 'omics can provide in general, the discovery of molecular initiating events and molecular pathways for adverse outcome pathways (AOPs) is promoted. While epigenetic modifications are likely primary molecular initiating events as per their role in constraining gene expression, there are still important challenges to overcome in order to incorporate epigenomics into regulatory frameworks, especially because consistent links to effects at the level of the phenotype (and then to higher levels of biological organization for improved ecological relevance) are yet to be established, as well as are trends and parallels across species. This is the foundation of the EPIBOOST research, a project addressing environmental epigenetics concerning aquatic organisms and model pollutants. EPIBOOST will focus on AOPs initiated by DNA methylation in model freshwater and marine organisms (microalgae, microcrustaceans, and fish), exposed to three model chemicals representing persistent or emerging threats nowadays or in the near future (metals, antibiotics, and toxins). Briefly, samples will be screened for global methylation and analysed via whole-genome bisulfite sequencing. Gene expression will be assessed via targeted or untargeted approaches to understand whether gene expression correlates to the changes in the epigenome. Phenotypic effects ranging from the subcellular to the population level will be assessed in parallel concerning specific (e.g., photosynthetic efficiency, neurotoxicity) and more general (e.g., growth rates, behaviour) responses to stress. Such a comprehensive approach will allow a meaningful insight on the AOPs for the selected model contaminants in the aquatic compartment, and particularly support the validation of epigenetic modifications as robust molecular initiating events.

#### **1.05.P-Th013 Time- and Dose-Dependent DNA Methylation Changes in Earthworms Exposed to Cadmium: Genome Wide and Gene-Specific Epigenetic Perspective**

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The heavy metal cadmium (Cd) can cause DNA hypermethylation, which has already been demonstrated in many invertebrates, including earthworms. However, detailed characterisation of DNA methylation landscape upon Cd exposure, as well as mechanisms behind the DNA hypermethylation, are still largely unexplored. Therefore, in this study, we used the earthworm model *Lumbricus terrestris* to explore time- and dose-dependent effects of Cd on global, genome wide, and gene-specific DNA methylation and its underlying mechanisms. Earthworms were exposed to two Cd concentrations (10 and 25 mg/kg) for a period of 12 weeks and sampled at several time points (1, 2, 4, 12 weeks). Global cytosine hypermethylation was revealed using specific antibodies in dot blots, whereas bsRADseq was used to determine genome wide DNA methylation changes and pinpoint specific genomic regions mostly affected by Cd exposure. To study the underlying mechanisms, we determined the gene expression as well as the activity of DNA methylation and demethylation components like DNA methyltransferases (DNMT1 and 3), and ten-eleven translocation (TET) genes. However, neither gene expression nor DNMT and TET enzyme activity showed significant differences in the Cd exposure groups. More specifically, we focused on the gene body methylation (gbm) of metallothionein 2 (MT2), one of the most important Cd detoxification proteins. Using bisulfite conversion and sequencing we were able to detect some changes in MT2 gbm; however, these changes could not be correlated to MT2 gene expression. The results of this study demonstrate the importance of studying epigenetic marks and underlying mechanisms in organisms under pollution pressure and point to missing links, which need to be further explored.

#### **1.05.P-Th014 Multigenerational DNA Methylation Patterns Following Copper Exposure in *Daphnia*: The Role of Exposure History**

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Epigenetic mechanisms are moving to the forefront of environmental sciences because of their key role in shaping gene-environment interactions, phenotypic plasticity, and evolutionary responses. Specifically, environmentally induced DNA methylation changes are known to shape biological responses to chemical contamination, and the transgenerational inheritance of methylation marks across nonexposed generations has been confirmed in invertebrate and vertebrate species. Here, we focused on the freshwater invertebrate *Daphnia magna*, with the goal of further understanding the involvement of DNA methylation in their response and eventual adaptation to copper contamination. To do so, we specifically tested the hypothesis that different histories



of exposure to Cu could determine different epigenetic signatures, which would be carried on into nonexposed generations. Accordingly, daphnids with different histories of past exposure to Cu were exposed to toxic levels of the metal for one generation (F0) and then monitored for three subsequent unexposed generations (F1, F2, and F3), with copper-induced DNA methylation changes, their potential transgenerational inheritance, and life-history traits being recorded. Overall, DNA methylation changes targeted important genes for counteracting the effects of metals and oxidative stress, including *dynein light chain*, *ribosomal kinase*, and *nuclear fragile X mental retardation-interacting protein*. Also, contrasting overall and gene-specific methylation responses were observed in organisms differing in their exposure history to Cu, with different transgenerational methylation responses being also identified among the two groups, without apparent life-history costs. Overall, results confirmed the capacity of Cu to promote DNA methylation transgenerational inheritance in a manner related explicitly to the history of exposure, thereby highlighting the potential for the development and incorporation of epigenetic biomarkers in risk assessment frameworks.

**1.05.P-Th015 Transgenerational Transcriptional Responses in *Daphnia magna*: The Role of Copper Exposure History**  
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Chemical exposures can extend their effects beyond “directly” exposed generations and influence the offspring response to future stressors. Thus, the establishment of transgenerational effects is a powerful phenomenon holding beyond exposure periods. Here, we assessed the transgenerational effects after Cu exposure in the invertebrate *D. magna* at the transcriptional level, while also evaluating the influence of acclimation history on such responses. To do so, daphnids with different histories of exposure, i.e., organisms acclimated in a Cu-enriched medium vs. blank ASTM for three generations, were exposed for one generation (F0) to an elevated concentration of the metal, and then the transcriptional response from F0 and F3 (F3 is the first truly unexposed generation) was inspected by real-time PCR employing an array with 41 genes (involved in detoxification and antioxidant response, DNA damage repair, circadian rhythm, and epigenetic regulation). Results showed that daphnids with different histories of exposure to Cu presented distinct transcriptional profiles at F0 and F3, with nonacclimated organisms showing higher modulation on gene expression. Despite the different exposure histories, histone modifier genes were always found to be transcriptionally altered in F0 and F3, suggesting the involvement of histone modifications in the response of *D. magna* to metal exposure. Besides, markedly distinct transgenerational transcriptional responses were found between the two groups of organisms, with nonacclimated daphnids showing a greater number of genes with modulated expression. Overall, results confirmed the influence of exposure history on transcriptional responses, and the ability of metals to determine transgenerational transcriptional responses across non-exposed generations.

**1.05.P-Th016 Marine Pollutant Tributyltin Affects DNA Methylation and Fitness of Banded Murex (*Hexaplex trunculus*) Populations**

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 Banded murex, *Hexaplex trunculus*, is a marine gastropod whose reproductive fitness can be severely affected by very low concentrations of antifouling compound tributyl-tin (TBT). Tributyl-tin has a strong xenoandrogen impact on snails, causing development of imposex (e.g., superimposition of male sexual characteristic in females), thereby affecting the fitness of entire populations. Tributyl-tin is also known as a DNA demethylating agent and an obesogenic factor. The aim of this study was to unravel the interactions between TBT bioaccumulation, phenotypic responses, and epigenetic and genetic endpoints in native populations of murex snail *H. trunculus*. Seven populations inhabiting environments along the pollution gradient were sampled in the coastal eastern Adriatic. Those included sites of intense marine traffic and boat maintenance activity, and sites with low anthropogenic impact. Populations inhabiting intermediately and highly polluted sites exhibited higher TBT burden, higher incidence of imposex, and higher wet mass of snails than populations on low polluted sites. Other morphometric traits and cellular biomarker responses did not show clear differentiation among populations in relation to the marine traffic/pollution intensity. Analysis of methylation sensitive amplification polymorphism (MSAP) revealed environmentally driven population differentiation and higher epigenetic than genetic within-population diversity. Moreover, decrease in genome-wide DNA methylation coincided with the imposex level as well as with the snail mass, suggesting epigenetic background of animal phenotypic response.

**1.05.P-Th017 Genotoxicity and Epigenotoxicity of CMIT/MIT on *Daphnia magna*: Trans- and Multigenerational Effects**  
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 CMIT/MIT (the mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) is the biocide consistently detected in the aquatic environment due to its broad-spectrum usage in various industrial fields. The ecotoxicological information of CMIT/MIT is very limited to within-generational toxicity evaluation, though multigenerational exposure frequently occurred. Furthermore, despite a possible role of epigenetics on genotoxicity, their relationship is poorly known in the ecotoxicological context. For that reason, we investigated whether parental (PE) and multigenerational exposure (ME) of CMIT/MIT led trans- and/or multigenerational effects by measuring various phenotypic biomarkers over four consecutive generations. Genotoxicity and epigenotoxicity of CMIT/MIT in *Daphnia magna* were also examined using the Comet assay and global DNA methylation. Our results showed the deleterious effects of CMIT/MIT at various endpoints of *Daphnia*, which had experienced the different exposure histories. Parental effects in the PE scenario can be transgenerational or recovered after the termination of exposure.

More interestingly, the ME could result in acclimatory/defensive responses. It was also found that changes in DNA damage were closely associated with the altered reproduction of daphnid, but their possible relationship with global DNA methylation was not found. Overall, this study provides the ecotoxicological information of CMIT/MIT from multifaceted endpoints and aids in understanding of multigenerational phenomena under the CMIT/MIT exposure. It also emphasizes the consideration of exposure duration and multigenerational observation in the evaluation of ecotoxicity, in terms of risk management for widely used biocides, such as CMIT/MIT.

#### **1.05.P-Th018 Investigation of Inter- and Intraspecific Sensitivity to CMIT/MIT in *Daphnia magna* and *pulex* Using Epigenetics and Proteomics Analysis**

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The adaptive response of organisms to environmental change involves both genomic and epigenomic components. Adaptation, whether genetically or/and epigenetically derived, underpins the sensitivity and tolerance of exposed organisms. Therefore, adaptation needs to be considered in evaluating the adverse outcomes induced by toxicants. This study investigates variation in sensitivity and epigenetic responses to chemical exposure using the strains stemming from different *Daphnia* populations. To this end, we assessed the comparative toxicity of a mixture of 5-chloro-2-methyl-4-isothiazoline-3-one and 2-methyl-4-isothiazolin-3-one (CMIT/MIT), an isothiazolinone biocide used in various types of consumer products, by measuring mortality, reproduction, physiological traits, global DNA methylation, and proteomic expression. The results showed a more significant variation in sensitivity to CMIT/MIT between conspecific strains (*Daphnia pulex* Rennes [DPR] vs. *Daphnia pulex* Alsace [DPA]) than between congeneric species (*Daphnia magna* INERIS clone A [DMI] vs. *Daphnia pulex* Rennes [DPR]). Furthermore, under control conditions, DPR (the strain most sensitive to CMIT/MIT) had a larger body size, a higher heart rate, and a higher level of global DNA methylation than its counterpart (DPA). Interestingly, the trend of global DNA methylation level alteration differed between the two strains. We also observed the strain-specific patterns of CMIT/MIT-induced differential protein expression, suggesting the role of protein alteration and associated pathways in the observed difference in susceptibility to CMIT/MIT. Collectively, our results indicate that epigenetic changes can contribute to intraspecific variation, and this variation at multiple levels should be considered in the evaluation of the ecotoxicity of biocide chemicals.

#### **1.05.P-Th019 Mechanisms of Nonlethal Heat Shock Induced Cross-Tolerance to Hydrogen Peroxide in Two Strains of Monogonont Rotifer *Brachionus plicatilis* Species Complex**

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Temperature is one of the main environmental factors influencing the presence and performance of organisms in aquatic ecosystems and affecting their survival and growth in aquaculture. Rotifer *Brachionus* sp. is a key species for aquaculture sustainability and, thus, the possibility to induce phenotypic traits that allow the organisms to cope with environmental stress poses a great advantage. It has been observed in several organisms, including in *Brachionus* sp., that short exposures to high temperatures lead to molecular changes (e.g., induction of heat-shock proteins, epigenetic modifications), resulting in induced thermotolerance. Therefore, this study aimed to evaluate the potential of nonlethal heat shock (NLHS) to also induce tolerance to other stressors, in two strains of rotifers belonging to the *Brachionus plicatilis* species complex. Exposure of organisms to 42°C for 30 min, followed by a recovery at 25°C for 8 h demonstrated to successfully increase tolerance to subsequent exposures to high salinity and hydrogen peroxide for one of the strains, and cadmium chloride for both strains. The mechanisms underlying the cross-tolerance effect to hydrogen peroxide after NLHS were further studied. Results showed an up-regulation of superoxide dismutase gene and higher levels of HSP70 in rotifers previously exposed to NLHS in the strain where cross-tolerance was successfully achieved. Moreover, epigenetic modifications were also observed. Genes encoding histone lysine methyltransferase (*KMT3E*), demethylase (*KDMI*), and acetyltransferase (*KAT6*) were significantly up-regulated, and the levels of histone H3 acetylation were also increased in heat shocked rotifers during the subsequent exposure to hydrogen peroxide. There was also the indication that heat shocked rotifers had higher histone H3 lysine methylation and acetylation levels, and lower levels of serine phosphorylation than control rotifers (without the NLHS) when exposed to hydrogen peroxide. However, levels of histone H3 acetylation decreased to control values before the end of the experiment (18 h exposure to H<sub>2</sub>O<sub>2</sub>), suggesting that the epigenetic effects observed may be transient and some more cycles of NLHS may be needed to promote a persistent effect. This study showed that the exposure to an environmental stressor (temperature) was able to induce changes in rotifer's epigenetic markers, resulting in positive phenotypic outcomes.

#### **1.06.A Fish Model Species in Human and Environmental Toxicology**

##### **1.06.A.T-01 Molecular and Cellular Effects Underlying Insecticide-Induced Neurobehavioural Alterations in Zebrafish Larvae**

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Molecular targets of insecticides are similar in insects and vertebrates. Insecticides thus pose a risk of potential neurotoxicity to aquatic vertebrates, such as fish. Neurotoxic effects often manifest as an alteration in an organism's behaviour. Hence, behavioural endpoints are used to assess the neurotoxicity of chemicals. A frequently used method is the zebrafish light-dark

transition (LDT) test, which measures the larvae's locomotion in response to light changes. Despite the popularity of the LDT, knowledge gaps exist regarding the underlying mechanisms that cause neurobehavioural effects. Therefore, this study aimed at investigating the links between insecticide-induced locomotor defects and (i) cellular changes in neuromuscular components (NMCs) and (ii) molecular changes such as mRNA expression of genes related to NMCs, as well as neurotransmitter concentrations. The locomotor behaviour of zebrafish larvae was measured after six-day exposure to two sublethal insecticide concentrations. Effects on NMCs were analyzed with fluorescence labelling and a birefringence approach. Exposure to the tested organophosphate (OP), carbamate (CBM), and neonicotinoid (NN) insecticides led to reduction in locomotion, while no effect was observed for the pyridine-derivatives. The other tested endpoints showed a different effect pattern for almost all insecticides. Among the NMCs, only the CBM methomyl and the NN imidacloprid affected motor axon growth. Furthermore, methomyl and the NN thiacloprid affected muscle integrity. The *ache* (acetylcholinesterase) gene was upregulated after exposure to most OPs and CBMs, while *smyd1b*, which plays a role in muscle development, was upregulated only after methomyl exposure. Metabolomics analyses showed that the acetylcholine concentration increased in fish exposed to methomyl, while glutamine and histidine concentrations were reduced in methomyl and thiacloprid-exposed fish, respectively. Kynurenine was increased after thiacloprid exposure, suggesting acute stress in these treatments. Our results demonstrate that locomotion is an integrative endpoint, caused by multiple underlying effects. By investigating these effects on various levels of biological organization, this study improves the mechanistic understanding of insecticide-induced neurobehavioural effects.

#### **1.06.A.T-02 Identification of Effect-Biomarkers for Vascular Disruptors as an Endpoint to Predict Developmental Toxicity Using Zebrafish Embryos**

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Inhibition of angiogenesis is an important mode of action for teratogenic effect of chemicals and drugs. Teratogenic drugs such as thalidomide but also environmental chemicals (herbicides, fungicides, and pesticides) have been shown to interfere with angiogenesis. Therefore, methods for the detection of angiogenesis inhibition by chemicals as an indicator of teratogenic effects form an important component for the integrated assessment of chemicals using alternative 3R-compliant approaches. The zebrafish embryo represents such an alternative model that offers the complexity of developmental differentiation of an entire organism while allowing for small-scale and high-throughput screening. Additionally, the transparency of its body allows for the application of imaging techniques as a screening method for chemical-induced morphological effects. Here we present a novel automated imaging-based method to detect angiogenesis inhibition. By subtracting video frames, we were able to identify the location and number of blood vessels according to moving blood cells. In order to identify a suitable model compound and exposure scenario for subsequent transcriptome analysis zebrafish embryos were exposed to various tyrosine kinase inhibitors (SU4312, SU5416, sorafenib, and PTK787). By video subtraction we confirmed that the compounds provoke concentration-dependent angiogenesis inhibition, with highest specificity for exposures starting at 24 h postfertilization. Based on the comparison of specificity and lethality, we selected SU4312 as a model compound, to perform concentration- and time-resolved microarrays. The final aim is to screen chemical induced vascular disruption by noninvasive, high-throughput video imaging and molecular biomarkers specific for anti-angiogenesis.

#### **1.06.A.T-03 Zebrafish Early Life Stages in the Study of Metabolic Disruption: The Case of Paraoxonase 1**

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Endocrine disrupting chemicals (EDCs) have been investigated for their disruptive effects on steroid hormone pathways. Some EDCs such as bisphenol A (BPA) have also been shown to disrupt energy metabolism, which could result in adverse health effects. Currently, the underlying mechanisms are not fully understood and there is an urgent need for assays to characterize these metabolic disrupting compounds (MDCs). Two different assays were developed using different life stages of the zebrafish. In each assay, zebrafish were studied in different metabolic and toxicological scenarios to investigate the role of paraoxonase 1 (PON1), an antioxidant enzyme associated to metabolic disorders by genome-wide association studies, in metabolic disruption. In the first assay, 14-day-old larvae were used to study the effects of metabolic disruption on PON1. Metabolic disruption was induced by a five-day feeding regime with a custom Western diet and aquatic exposure to different doses (200 µg/L or 2000 µg/L) of a known MDC, BPA. The Western diet feeding regime resulted in a nonsignificant increase of weight, length, and oxidative stress. The addition of BPA exposure on top of the Western diet resulted in a significantly increased weight, length, and oxidative stress, indicating that the metabolic disrupting effects of BPA further exacerbated the metabolic changes induced by the diet. PON1 activities increased either in all Western diet groups, or in a combination of the Western diet and exposure to 2000 µg/L BPA in response to the diet- and dose-dependently increasing oxidative stress. In conclusion, this assay successfully studied metabolic disruption, and was able to identify that PON1 likely performed part of the antioxidant response to the increasing oxidative stress caused by metabolic disruption.

In zebrafish embryos, we investigated which effects indicative of metabolic disruption can already be measured at 120 h postfertilization (hpf). Until this age, zebrafish are considered a new approach methodology (NAM), an alternative to animal testing. Embryos were exposed from 2 to 120 hpf to different MDCs including BPA, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) agonist rosiglitazone and the PPAR $\gamma$  antagonist T0070907. Embryos showed dose-dependent increases in yolk size for all tested compounds. The embryonic assay can quickly identify MDCs using a NAM approach, whilst the larval assay allows for the study of MDCs in interaction with a dietary component.

#### 1.06.A.T-04 A Battery of Behaviour-Based Assays in Larval Zebrafish to Elucidate Acute and Developmental Neurotoxicity Mechanisms

**David Leuthold<sup>1</sup>**, Nadia Katherine Herold<sup>2</sup> and Tamara Tal<sup>3</sup>, (1)Chemicals in the Environment Research Section, Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany, (2)Bioanalytical Ecotoxicology, Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany, (3)Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany. Approximately one in six children is diagnosed with a developmental disability including learning and intellectual disability. While causes are often elusive, chemical exposure during prenatal and early postnatal development can demonstrably interfere with neurodevelopment. However, due to extensive time, cost, and animal number requirements of developmental neurotoxicity (DNT) guideline studies in rats, the potential hazard of most chemicals remains unknown. To fill this gap, we devised a battery of 11 automated behaviour assays in larval zebrafish, a 3R-compliant model amenable to higher-throughput chemical screens. The battery captures various stereotypical visual and acoustic behaviours including habituation, a form of nonassociative learning. We hypothesized that behaviour-rich phenotyping of chemicals provides a comprehensive readout of potential adverse outcome pathways (AOPs) that dictate a broad range of events and interactions that orchestrate nervous system development and function. According to the AOP “Impairment of Learning and Memory,” we focused on the NMDA receptor (NMDAR) as a target of chemical exposure. In an initial step, the DNT NAM was evaluated against seven drugs with distinct mechanisms including NMDA and GABA receptor (GABAR) antagonism. In line with effects reported in rats, exposure to the prototypical NMDAR antagonist MK-801 caused a deficit in habituation learning. Surprisingly, cluster analysis revealed high phenotypic similarity to the GABAR antagonist picrotoxin, pointing to a potential second mechanism of MK-801, GABAR antagonism. The battery was further evaluated against a set of 10 USEPA ToxCast chemicals positive for *ex vivo* NMDAR antagonism. In addition to methadone, acute exposure to chlorophene, a biocide and preservative in cosmetics, was confirmed to affect habituation learning *in vivo*. Defective habituation learning was also observed in a developmental exposure paradigm (0.25-4 d postfertilization), indicating chlorophene-induced DNT. Strikingly, in addition to an NMDAR-mediated learning deficit, chlorophene exposure caused “paradoxical excitation,” a phenomenon linked to GABAR agonism. Pharmacological intervention using GABAR antagonist picrotoxin blocked chlorophene-induced sedation, substantiating its gabaergic interaction. In summary, these nonexhaustive examples highlight the capacity of this behaviour-based NAM to illuminate AOPs, to reduce/replace existing *in vivo* approaches and to accelerate DNT research.

#### 1.06.A.T-05 A New Test System for Assessing Toxicity of Chemicals with Low Water Solubility: Dechlorane Plus as a Case Study

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Global regulatory authorities require chemical toxicity data from vertebrate test systems, including fish, to identify, mitigate, and manage any adverse environmental effects arising from their use. The current regulatory tests for environmental chemical hazard assessment are based on waterborne exposures. However, many potentially toxic chemicals are highly hydrophobic/lipophilic (water-octanol coefficient –  $K_{ow}$  –  $>6$ ) and present a practical challenge for conducting aquatic exposures (high volumes of solvents, welfare issues), especially at early life stages. To date only one regulatory test guideline, the fish bioaccumulation test (OECD TG305), offers a dietary chemical delivery option. Although TG305 does not aim to assess toxicity assessment, the existing guidance can be easily adapted to most aquatic tests, including for endocrine disrupting chemicals (EDCs).

We designed such a test system using Dechlorane Plus (DP), a very strongly hydrophobic ( $\log K_{ow} > 6$ ), persistent and bioaccumulative chemical with poorly understood toxicity (at the time, DP was under pending decision to enter the POP list) and potential for endocrine disruption. In order to capture potential effects at early life stages, including embryos that do not feed, we started the dietary exposure of the parent generation, similarly to TG229/230 and follow-up with the Fish Embryo Toxicity Test (TG236). There were four treatment groups: Control (0  $\mu\text{g/g}$  DP), Low (0.1  $\mu\text{g/g}$  DP), Medium (1  $\mu\text{g/g}$  DP), and High (10  $\mu\text{g/g}$  DP). This bespoke test aimed to obtain a comprehensive toxicity assessment of DP over two generations of fish, building on the way resources for developing NAMs (new approach methodologies) by focusing on the zebrafish embryo responses with the aim of developing predictive markers of exposure/toxicity and ultimately, an adverse outcome pathway (AOP).

#### 1.06.B Fish Model Species in Human and Environmental Toxicology

##### 1.06.B.T-01 Ecosafety Evaluation of Biogenic Matrices for Agricultural Reuse Using Zebrafish Embryos

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The use of biogenic matrices as fertilizers and amendments is considered a good solution to improve soil properties and resilience. Nevertheless, there are growing concerns related to their potential hazard to ecosystems and human health, due to the potential presence of harmful organic pollutants, metals, and microbiological contaminants. For this reason, there is an urgent need to develop effect-based tools to guarantee their safe application into the environment. In this scenario, this project aimed to

compare the ecotoxicity of four different types of biogenic matrices: manure (M1 and M2), compost (C), bovine manure digestate (D), and sewage sludge (S1–S2–S3), which all have fertilizing properties, using zebrafish (*Danio rerio*) embryos. To this aim, we assessed acute toxicity and sublethal effects as behavioural alterations, metabolic disruption, and neurotoxicity of elutriates (at dilutions: 1:100, 1:50, 1:25, and 1:10) as well as of the whole matrix (through contact assay, at a concentration of 1:300). All matrices, except for S2 and S3, displayed 100% of embryotoxicity at the dilution 1:10 at 120 hpf, whereas no relevant embryotoxicity was induced at 1:50 and 1:100 dilutions, except for S1 and D. Concerning metabolic endpoints, the Seahorse analysis showed that all the samples have boosted both basal oxygen consumption and maximal respiration in comparison to control embryos. Regarding behaviour, S2 displayed increased motility compared to the control at all the tested dilutions and S3 at the dilution of 1:100 and 1:50, while no significant differences were observed in the other matrices. Finally, acetylcholinesterase activity displayed no statistically significant variation regardless of the matrix or the dilution. In conclusion, our results highlighted that these biogenic matrices, at high concentrations, may affect biochemical and physiological functions of zebrafish embryos at a similar extent (S2 and S3 being the best substrates, while S1 and D the worst). In particular, they can alter swimming performance, metabolism, and embryo development. The integration of our data with results related to chemical characterization and other bioassays, still ongoing, will provide an overall and representative view of the real features of these matrices, which shall be carefully evaluated also in view of the land-application conditions.

#### 1.06.B.T-02 Integrative Assessment of Ocean Pollution Impacts in Blue Sharks (*Prionace glauca*)

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Blue sharks (*Prionace glauca*) are one of the most widely distributed and caught shark species worldwide. Although an increasing number of studies have been tracking the concentrations of pollutants in the tissues of these sharks, much less is known about how they can affect organisms' health. Therefore, this study aimed to further understand how different types and levels of contamination can impact blue sharks' general fitness, with the ultimate goal of developing a good and reliable set of tools to assess sharks' health and biomonitor oceanic environments. For this goal, a total of 60 sharks were opportunistically captured as by-catch aboard a fishing vessel, and sampled for the analyses of contaminant levels (metals and Persistent Organic Pollutants [POPs]), stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), and biological responses in different tissues: 1) at molecular level – whole transcriptome sequencing analysis (RNAseq); 2) at cellular level – fatty acid (FA) profile and oxidative stress related biomarkers; 3) at tissue level – histopathological alterations. Results showed that sharks captured closer to the continental shore exhibited higher levels of contaminants in comparison to the ones captured in a more pelagic environment, being the accumulation mostly influenced by their geographical distribution, rather than sex, size, or trophic level of their prey. Transcriptomic data showed several differentially expressed genes in each of the four sampled tissues when comparing the individuals caught in the two geographical areas, and strong correlations with metals (e.g., fatty acid metabolism) and POPs (e.g., immunoglobulins). The liver FA profile revealed that female sharks seem to be the most affected group, presenting significantly lower levels of the main classes of FAs in individuals caught in the coastal area, explained by strong negative correlations with both metals and POPs. Similar zone-related differences were found in biomarker responses and strong positive correlations between, e.g., As, PCBs, and PBDEs, and DNA damage. Histopathological lesions were also observed in the sampled tissues, having overall higher severity levels with higher contaminant concentrations. These results show clear indications of higher stress, cellular damage, and tissue injuries in organisms with higher pollutant levels, highlighting the suitability of this species and these tools to monitor contamination and its effects in different areas of the Atlantic, under real exposure scenarios.

#### 1.06.B.T-03 Probiotic Administration Counteracts Bisphenol A-Induced Toxicity at Brain-Gut-Microbiome Axis Level in *Danio rerio* Adults

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Several studies demonstrated that Bisphenol A (BPA) possesses toxicity at brain and intestinal levels both in mammalian and teleost species. The pollutant induced toxicity at the intestinal level could cause damage at the central level or vice versa through the brain-gut-microbiome axis. Probiotics beneficial effects were observed in numerous vertebrate species and could represent a tool to decrease the BPA induced alterations. In this regard, SLAB51 is a probiotic formulation which ameliorated gastrointestinal pathologies and showed neuroprotective ability in diverse veterinary trials. The ability of SLAB51 to counteract BPA toxicity was here investigated on brain-gut-microbiome axis in zebrafish adults in a chronic (28 days) exposure to 10  $\mu\text{g/L}$  BPA plus SLAB51 ( $10^9$  CFU/g) (BPA+P); results were compared with those of an untreated control (C) and of those treated with BPA or SLAB51 (P) alone. At brain level, while females reported only an increase of microvessel alteration, males were more impacted by BPA toxicity since neuronal degeneration, microvessel alteration, and perivascular edema were present. The TUNEL assay indicates an increase of cell death in the brain in both male and female fish exposed to BPA associated with the upregulation of neurodegeneration markers such as TAU aggregates and glial fibrillary acidic protein (GFAP). In BPA+P treated fish, the same biomarkers presented levels similar to C ones, demonstrating the counteracting effects of probiotics on BPA toxicity. In the gut,

BPA induced a reduction of intestinal fold length and an increase of the lamina propria thickness similar in both the male and female gut, while a reduction of intestinal muscle thickness was only observed in female fish. At the transcript level, BPA reduced *litaf* in both sexes and *il-1 $\beta$*  and *il-10* only in females, suggesting the contaminant ability to impair the immune system. In BPA+P group no gut alterations were found. The gut microbiota analysis evidenced in BPA and BPA+P the presence of *Pseudomonadales*, which can use BPA as substrate for growing, while the *Cetobacterium*, a vitamin B12 producing bacteria, was detected in P and BPA+P exposed fish, this vitamin being useful for improving organism health status. These results show for the first time the ability of SLAB51 to counteract BPA toxicity in zebrafish gut and brain, suggesting the possible use of this formulation in the treatment of toxicity induced by a chronic exposure to environmental BPA levels and possibly other endocrine disruptors.

**1.06.B.T-04 Widespread Psychoactive Pollutant Disrupts Fish Circadian Activity Rhythms** *Hung Tan<sup>1</sup>, Jake Martin<sup>1,2</sup>, Lesley A. Alton<sup>1</sup>, John A. Lesku<sup>3,4</sup> and Bob B.M. Wong<sup>1</sup>*, (1)School of Biological Sciences, Monash University, Australia, (2)Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences (SLU), Sweden, (3)School of Agricultural, Biomedicine and Environment, La Trobe University, Australia, (4)Research Centre for Future Landscapes, La Trobe University, Australia

Pharmaceutical pollution is an emerging driver of global change, with the capacity to alter key behavioural and physiological traits in exposed animals. Antidepressants are among the most commonly detected pharmaceuticals in the environment. Despite well-documented pharmacological effects of antidepressants on sleep in humans and other vertebrates, very little is known about their ecologically relevant impacts as pollutants on nontarget wildlife. Accordingly, we investigated the effects of acute three-day exposure of Eastern mosquitofish (*Gambusia holbrooki*) to current field-realistic levels (nominal concentrations: 30 ng/L and 300 ng/L) of the widespread psychoactive pollutant, fluoxetine, on circadian activity patterns and restfulness, as indicators of disruptions to sleep. Using automated tracking software, we tracked approximately 10 000 hours of behavioural video footage, quantifying the activity levels of 140 adult individuals across the full 72-h exposure period. Here, we show that exposure to fluoxetine disrupted diel activity patterns, which was driven by augmentation of daytime inactivity. Specifically, unexposed control fish were markedly diurnal, swimming farther during the day and exhibiting longer periods and more bouts of inactivity at night. However, in fluoxetine-exposed fish, this natural diel rhythm was eroded, with no differences in activity or restfulness observed between the day and night. As a misalignment in the circadian rhythm has been shown to adversely affect fecundity and lifespan in animals, our findings reveal a potentially serious threat to the survival and reproductive success of pollutant-exposed wildlife.

**1.06.P-Th020 Elemental Concentrations in the Invasive Australian Redclaw Crayfish, *Cherax quadricarinatus*, Pose Human Health Risks in the Largest Floodplain System of South Africa**

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The Australian redclaw crayfish, *Cherax quadricarinatus*, has been introduced globally for aquaculture and aquarium trade purposes, and invasive wild populations have established in several African countries, including South Africa, Eswatini, Zambia, and Mozambique. Due to the growth in population numbers of these invasive crayfish, several low-income and rural communities are utilising them as an inexpensive, easily accessible protein source. Although this crayfish has been introduced on a global scale, limited research has been done on element accumulation in this species, and the risks that consumption may pose to humans. This study focused on elemental accumulation (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) in the redclaw crayfish from two rivers of the largest floodplain system of South Africa, the Phongolo River floodplain (PRF). The carcinogenic and noncarcinogenic human health risks associated with its consumption were calculated. The element accumulation order in the PRF was Zn > Cu > Pb > Cr > As > Ni > Hg > Cd, whilst the highest concentrations of As, Cd, Cr, Hg, and Pb were recorded in the Phongolo River. High concentrations of Cu, Ni, and Zn were associated with the Usuthu River tributary. Carcinogenic health risks were associated with consumption of the crayfish from all of the sites for As, Cr, and Ni, where 19, 10, and 6 out of 100 000 people could develop cancer from exposure to these elements, respectively. Concentrations of Pb also posed a level of concern for cancer risk at all of the sites. The Hazard Quotient of *C. quadricarinatus* collected in the Phongolo River were 1.7 for As and 2.2 for Hg indicating a high probability of adverse noncarcinogenic health risk. These results highlight that wild populations of invasive redclaw crayfish do not only pose a threat on aquatic ecosystems, but the consumption of these crayfish from the PRF could potentially also cause several carcinogenic and noncarcinogenic human health risks when consumed.

**1.06.P-Th021 Lipidic Effects of Metals in Liver of Blue Shark (*Prionace glauca*) and Small-Spotted Catshark (*Scyliorhinus canicula*)**

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As apex predators, sharks play a key role regarding the health and balance of marine ecosystems. However, these are very susceptible species to increasing oceanic pollutants exposure and bioaccumulation during their long lifespans. The liver is one of the largest organs in these species, extremely important in biological processes such as detoxification or buoyancy. It is composed of several classes of lipids and, consequently, an enormous diversity of fatty acids that are important as energy resources, membrane constituents, or as metabolic and signalling mediators. There are several studies addressing lipids and fatty acids as promising indicators of environmental contaminants' effects in coastal or deep-sea organisms but these studies in sharks are still scarce. Since metals have been demonstrated to accumulate in muscle and liver tissues from several marine organisms, normally leading to lipid oxidation, the present work aimed at exploring how different metals accumulated in two shark species (*Prionace*

*glauca* and *Scyliorhinus canicula*) could be associated to changes in different classes of fatty acids (FA) in their livers. To this purpose, liver samples were collected from approximately 60 individuals from each species, and their FA profile and content analysed and correlated with the metal levels accumulated in their tissues, having also in consideration biotic and abiotic factors such as age, gender, and geographical distribution. Elements such as Al, AS, Fe, Hg, Se, and Zn were detected for both species and results indicated general higher accumulation levels in *P. glauca* comparatively to *S. canicula*. Liver FA profile was found to be different between species, but also within species, considering maturation stage and gender. The analysis suggests that *P. glauca* females are the ones most influenced by the pollutant levels, with several FA classes being strongly and negatively correlated with the majority of the metals detected. However, a set of FAs were found with the same trend for both genders of the species (-trans and n9 FAs). In *S. canicula*, the elements that most influenced the variability of responses in FA profile were Hg, Fe, and Zn, with trans-palmitoleic, eicosenoic, and DHA FAs presenting significant correlations with those metals. Similar studies are essential for a better understanding of the impacts that environmental factors can cause on the physiology of these organisms and to infer about the possible ecological consequences of such impacts.

#### 1.06.P-Th043 Do Halogenated Environmental Toxins Cause Immunosuppression in Baltic Sea Salmon?

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The Baltic Sea is one of the most contaminated seas in the world, heavily polluted with numerous organohalogen compounds, causing large body burdens in Baltic Sea wildlife, such as Baltic Sea salmon (*Salmo salar*). Using the zebrafish embryo model (ZFE), we set out to test the hypothesis that exposure to organohalogen mixtures may contribute to the adverse health status observed among the salmon population, through impaired immune system function.

We identified 41 organohalogens in serum of Baltic Sea salmon and based on their relative prevalence and prior knowledge regarding toxicity, nine of these were selected and evaluated for their ZFE toxicity, toxicokinetic parameters, transcriptional responses, and lastly impact on the development and function of neutrophils and macrophages upon chemical and inflammatory stress.

Several dose-dependent morphological effects were observed, including scoliosis, edemas, reduced growth, and abolished swimbladder inflation. While the individual components of the mixtures caused different effect-profiles, most morphological effects appeared in an additive manner when exposed as a mixture. By performing RNAseq based transcriptomic profiling, the main effects identified were altered metabolic function and a suppressed immune system. This was further supported by studying ZFE reporters with fluorescently labelled immune cells, indicating both a reduced number of mature immune cells, as well as reduced function upon inflammatory stress.

Although the results need to be confirmed using relevant fish species, our data strongly indicate potential risks for immunotoxicity in wildlife fish exposed to these compounds. Moreover, the large difference in toxicokinetic properties observed for the compounds tested here, resulting in distinct internal-dose profiles over time, highlights the necessity of proper determination of external and internal concentrations for accurate ranking of toxic potency.

#### 1.06.P Fish Model Species in Human and Environmental Toxicology

##### 1.06.P-Th023 Neurobehaviour Changes of Adult Zebrafish Under Exposure to Chemical Ingredients in Commercial Fragrant Products

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The growing number of chemicals in commercial products raises concerns about unintended or intended exposure, negatively impacting human health and the environment. Through Association Rule Mining analysis, a total of 212 substances on the basis of the top 10% occurrence among 100 fragrance products, citronellol, geraniol, and their mixture were selected as the most suspicious substances in fragrance products. To investigate their potential in neurotoxicity we exposed 50-fold diluted no-effect concentrations (i.e., citronellol 0.2 µg/mL, geraniol 0.4 µg/mL, and a mixture of both substances) obtained from 96-h acute embryonic toxicity testing to zebrafish adults for 14 days. The photomotor response test (PMR), novel tank test (NTT), and T-maze test were conducted to measure neurobehavioural changes. In the NTT, exposure to citronellol and geraniol exerted a tendency to decrease in time and distance in the top area. Also, significant changes were observed in the PMR test, indicating that photomotor response was suppressed. In the T-maze test, there was no significant difference in the movement distance of the left and right zones in the exposed group, suggesting that memory ability was damaged. Neurotransmitter analysis indicated that the level of 5-hydroxyindoleacetic acid (5-HIAA) associated with depression was significantly decreased in the citronellol group and mixture group compared to the control. Also, a significant decrease in acetylcholine (ACh), which plays an important role in cognitive functions, was observed in the mixture group. In summary, our study shows that long-term exposure to low concentration of citronellol and geraniol, major ingredients of commercial fragrance products, had the potential for neurotoxicity in zebrafish, suggesting a cautionary use of fragrant chemicals.

##### 1.06.P-Th025 Utilizing a Population-Genetic Framework to Test for Gene-Environment Interactions Between Zebrafish Behaviour and Chemical Exposure

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Individuals within genetically diverse populations display broad susceptibility differences upon chemical exposures. Understanding the role of gene-environment interactions (GxE) in differential susceptibility to an expanding exposome is key to protecting public health. However, a chemical's potential to elicit GxE is often not considered during risk assessment. Previously, we have leveraged high-throughput zebrafish (*Danio rerio*) morphology screening data to reveal patterns of potential GxE effects. Here, using a population genetics framework, we apportioned variation in larval behaviour and gene expression in three different PFHxA environments via mixed-effect modeling to assess significance of GxE term. We estimated the intraclass correlation (ICC) between full siblings from different families using one-way random-effects model. We found a significant GxE effect upon PFHxA exposure in larval behaviour, and the ICC of behavioural responses in the PFHxA exposed population at the lower concentration was 43.7%, whilst that of the control population was 14.6%. Considering global gene expression data, a total of 3746 genes showed statistically significant GxE. By showing evidence that heritable genetics are directly affecting gene expression and behavioural susceptibility of individuals to PFHxA exposure, we demonstrate how standing genetic variation in a heterogeneous population such as ours can be leveraged to test for potential GxE.

#### **1.06.P-Th027 Novel Insight into the Mode of Action of Metabolic Endocrine Disruptors (MDCs) in the Developing Intestine Through the Use of a Transgenic *tg(cyp3a65:GFP)* Zebrafish Model**

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Metabolic endocrine disruptors (MDCs) have the ability to promote metabolic changes which could ultimately result in obesity, diabetes, and/or nonalcoholic fatty liver disease in humans. Significant advances have been made on the modes of action by which MDCs disrupt metabolic pathways but there are still needs to further study their modes of action on less investigated molecular and cellular pathways and develop relevant mechanism-based models. In this study, we developed and characterized a transgenic zebrafish (zf) model *tg(cyp3a65:GFP)* expressing Green Fluorescent Protein (GFP) under the control of the promoter of *cyp3a65*, ortholog of human *cyp3a*, which play a critical role in xenobiotic but also cholesterol metabolism. *In vivo* imaging and immunohistochemistry showed an early constitutive GFP expression during embryonic development starting at 72 h postfertilization (hpf) in enterocytes of the anterior part of the intestine. Mono-exposure of embryos from 72 to 120 hpf to zfPXR and zfAhR ligands (clotrimazole and TCDD, respectively) induced GFP after 24 h and 48 h of exposure, showing that these two transcriptional factors play a role in the regulation of *cyp3a65*. Further investigations showed that zfPXR and zfAhR act cooperatively to induce *cyp3a65* expression as coexposure experiments resulted in a higher induction as compared to single exposure experiment to zfPXR or zfAhR ligands. Furthermore, we showed that CH223191, an AhR antagonist, promoted the expression of *cyp3a65* but also the effect of clotrimazole on the *tg(cyp3a65:GFP)* model since both the basal and the clotrimazole-induced *cyp3a65* expression were higher when the AhR signaling pathway was blocked by CH223191.

Overall, we successfully developed a stable transgenic model allowing study of the the expression of a key target gene in the developing intestine of zf. We showed that the zfPXR and zfAhR are critical in the regulation of the *cyp3a65* gene and that these two signalling pathways interact in an ambivalent manner. While there is still a need to pursue this research to enhance our knowledge on the mechanism regulating *cyp3a65* in intestine, the *tg(cyp3a65:GFP)* zebrafish embryo represents a promising model to develop a mechanism-based bioassay to screen the activity of chemicals (which is currently under progress). The causal links between *cyp3a65* disruption and potential metabolic adverse effect(s) also represent a challenge for efficient use in a hazard assessment perspective.

#### **1.06.P-Th028 Evaluation of Developmental Toxicity, Immunotoxicity, and Endocrine Disruption Caused by Exposure to Triazines, Triazoles, and Short-Chain Per- and Polyfluoroalkyl Substances**

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Because persistent and mobile (PM) substances have a high probability to end up in drinking water, information on their hazards is crucial to keep drinking water safe for consumption. Many triazoles, triazines, and short-chain PFAS are persistent and mobile due to their specific molecular structures. Within the EU-H2020 project ZeroPM, *in vitro* toxicity profiles were determined for 16 triazoles, nine triazines, and 11 short-chain PFAS with respect to developmental toxicity, immunotoxicity, and endocrine disruption. Developmental toxicity was assessed in zebrafish embryos using a standardized scoring system. This general morphology score indicates a delay in embryonic development. The embryos were also scored for teratogenicity based on several tail and head malformations and edema. The No Observed Adverse Effect Concentration (NOAEC) of the developmental toxicity tests were tested in two immunotoxicity assays with zebrafish embryos. In the first test, the embryos were infected by yolk sac injection with fluorescently labeled *Mycobacterium marinum* and subsequently exposed to the chemicals of interest. At 96 hpf the fluorescence was measured as a proxy for bacterial load. The second immunotoxicity assay measured the migration of neutrophils and macrophages. Transgenic zebrafish with fluorescent neutrophils or macrophages were exposed to the chemicals of interest and their tailfin was amputated at 72 hpf. The number of macrophages and neutrophils at the site of amputation were counted after three hours. A lower number of phagocytes indicates that exposure to the chemical interferes with their migration. As tests for endocrine disruption, agonism, and antagonism toward the estrogen receptor (ER), an oogen receptor (AR) and thyroid



hormone receptor (TR) were assessed using reporter gene cell lines. Exposure to nine triazoles showed an antagonistic response toward the AR, with IC50s between 4.2 and 56  $\mu$ M. Additionally, two triazines showed antagonism toward the AR with IC50s near 100  $\mu$ M. Seven of the chemicals that antagonized the AR also showed an antagonistic response in the ER, while two others showed an agonistic response in the ER. Result from the *in vitro* toxicity profiling will be calculated into external benchmark concentrations, using QIVIVE models developed by ZeroPM partner Fraunhofer-ITEM. Comparison of these external benchmark concentrations to exposure estimates for PM substances modeled by ZeroPM partner TG Environment, will ultimately result in a risk prioritization tool for PM substances.

#### **1.06.P-Th029 *In vitro* and *in vivo* Experiments to Evaluate the Endocrine Disrupting Toxicity of DEHTP in Relation to the HPG and GH/IGFs Axis**

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Bis(2-ethylhexyl) phthalate (DEHP) is widely known to cause reproductive toxicity and endocrine disruption in animals and humans. In response to the restriction of DEHP, di-2-ethylhexyl terephthalate (DEHTP) has often been used as an alternative. In the present study, adult zebrafish pairs were exposed to various concentrations of DEHTP for 21 d, and the effects on reproduction, sex hormones, and transcription of the genes belonging to the hypothalamic-pituitary-gonad (HPG) axis were investigated. Using the H295R cell line, the potential endocrine disrupting effects of DEHTP were also assessed. The effects on development, growth hormones, and changes in expression of the genes related to growth hormone/insulin-like growth factors (GH/IGFs) axis were investigated after exposure to DEHTP in zebrafish embryos for 96 h. Egg production was significantly decreased at  $\geq 30$   $\mu$ g/L DEHTP, and plasma concentrations of testosterone (T) were significantly increased in female fish. In male fish, however, significant decreases of T were observed along with significant increase of E2 and up-regulation of *vitellogenin* transcript. This observation was consistent with increased E2 and enhanced expression of CYP19 mRNA of H295R cells. Body length and growth hormone levels were significantly reduced when zebrafish embryo were exposed to  $\geq 30$   $\mu$ g/L DEHTP, and transcription of genes related to GH/IGFs axis was significantly downregulated. Our observations showed that exposure to low level DEHTP could affect the feedback circuit of HPG axis and GH/IGFs and impair the reproduction as well as development.

#### **1.06.P-Th030 Screening for the Potential Disruption of the Thyroid or Estrogen-Receptor Signaling Pathway using the Zebrafish Embryo**

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The plethora of chemical substances and their mixtures pose an increasing risk to human and environmental health. A special cause of concern are endocrine-disrupting chemicals (EDCs) that interfere with an organism's normal functioning of the endocrine system. The main sources of EDCs in surface waters are industrial and municipal effluents that are discharged to the aquatic environment. Of particular interest are estrogenic EDCs (EEDCs) or substances that alter the thyroid signalling pathway due to their deleterious effects on the organism's metabolism, development, and reproduction. There is evidence for the multiple cross-talk between the hypothalamus-pituitary-thyroid (HPT) and hypothalamus-pituitary-gonadal (HPG) axis; however, adequate, cost-effective, and efficient tools are required to screen chemicals and their mixtures as EDCs.

Here, we present a parallel assessment of model substances to identify their potential to disrupt the thyroid- and/or estrogen-receptor signaling pathways by the use of the zebrafish model as a proof of concept. By the use of the transgenic fish lines tg(tg:mCherry) and the tg(cyp19a1b:GFP), zebrafish embryos were exposed to a set of known EDCs to assess the fluorescence levels of the thyroid gland and cerebral structures. By measurements of the reporters that are modulated by the promoter activity of the thyroglobulin or respective cyp19a1b brain aromatase, the enzyme which is responsible for the synthesis of estrogens from androgens, the tested compounds were classified. To identify potential downstream effects of the impaired endocrine system, a new liquid chromatography coupled to tandem mass spectrometry (HPLC/MS-MS) method was developed to quantify the thyroid hormone (TH) levels of T4, T3, 3,5-T2, and 3,3'-T2 in whole-embryo homogenates. Additionally, gene expression analyses for the thyroid- and estrogen-signalling pathway were performed using RT-qPCR applying known markers to assess the transcription levels of thyroid (*tsh $\beta$* , *tpo*, and/or *tg*) and estrogenicity-relevant (*vtg*, *esr1*, and/or *esr2a*) genes.

This content screening method improves the screening of chemicals for their endocrine disruption potential and to further characterize their Mode of Action (MoA). The joint assessment of the HPT- and HPG-axis helps to better understand the potential interference of chemicals in this crosstalk and advances the assessment of potential adverse outcomes.

#### **1.06.P-Th031 Transcriptomic Profiling of Clobetasol Propionate Induced Immunosuppression During a TLR-7-Dependent Immune Challenge in Zebrafish Embryos**

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These days, our environment inevitably has to face increasing pollution with chemicals, their active ingredients, and metabolites. To assess their potential hazard toward nontarget organisms and the ecosystem, the approval of new chemicals is subject to strict regulations and intensive testing. However, currently comprised endpoints do not comprehensively allow the identification and differentiation of modes of action and lack the ability to assess effects on certain biological key mechanisms. For instance, the

modulation of the immune system is still neglected, although its adverse impact at the scale of individuals, populations, and whole ecosystems is well confirmed. Reasonably, the complexity of the immune system and the lack of representative biomarkers hinder the development of reliable immunotoxic screening methods. To fill this gap, 'omics techniques represent promising tools, permitting a broad-viewed response analysis at the global level of gene expression from a single sample. With transcriptomic and proteomic analysis of chemically immunosuppressed and immune-challenged zebrafish embryos, our study aims to identify ecotoxicogenomic fingerprints and biomarkers for immune-related modes of action to promote the development of said screening methods. To trigger the desired effects, two pharmaceutical agents, clobetasol propionate (immunosuppressor) and imiquimod (immunostimulator), were used. Implementing imiquimod, an anti-tumoural agent with the potential to undesirably cause psoriasis, this study also aims to provide an alternative system for immunotoxic screening methods, increasing the predictability of immune-related side effects of new pharmaceutical agents in humans, beside its application in the ecotoxicological assessment. So far, pretests with clobetasol propionate have been completed, revealing 250 nM as an appropriate concentration to trigger an immunosuppression in a sublethal manner. Respective experiments for imiquimod are ongoing. With these determinations set, transcriptomic and proteomic profiles of immunosuppressed and subsequently immune-challenged zebrafish embryos will be assessed based on RNA sequencing and LCMS/MS data. Identified differentially expressed genes may serve as suitable biomarkers for an immunotoxicity assessment, while their anticipated implementation to current guidelines in the future will represent an important step toward a more comprehensive environmental hazard assessment.

#### 1.06.P-Th032 Evaluation of the Toxicity of Guanitoxin-Producing Cyanobacteria on *Danio rerio* Hepatocytes Culture (ZF-L)

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Human actions are increasingly degrading aquatic ecosystems. These alterations can modify the composition of the primary producers, promoting the excessive proliferation of microorganisms, including cyanobacteria. Cyanobacteria can produce secondary metabolites, such as guanitoxin, the only naturally occurring anticholinesterase organophosphate, with a potential effect similar to synthetic insecticides. This study, therefore, investigated the acute toxicity of guanitoxin-producing cyanobacteria *Sphaerospermopsis torques-reginae* (ITEP-024 strain) extracts in hepatocytes of *Danio rerio* (ZF-L cell line). Different concentrations (0, 1, 4, 8, 16, 31.25, 62.5, 125, 250, 500 mg/L) of the ITEP-024 aqueous and methanolic extracts were tested in *D. rerio* for 24 h. We evaluated the viability of zebrafish hepatocytes by the Alamar Blue assay (cytotoxicity indicator). The secondary metabolites produced by the ITEP-024 strain were analyzed by LC-MS/MS. The results showed an interaction between the aqueous extract containing guanitoxin and ZF-L cells, showing an influence on cell viability and being an indicator of cytotoxic effects. The assay proved to be a powerful tool for detecting the impact of cyanobacterial cells on the cell population. The values referring to cell viability showed that the concentration of 500 mg/L presented five times fewer intact cells than the negative control after a 24-h exposure. The EC(I)50<sub>24h</sub> was 100.2 mg/L. Analysis of LC-MS/MS indicated the guanitoxin presence in the aqueous extract of the ITEP-024 biomass. In conclusion, aqueous extracts of *S. torques-reginae* showed damage in the cell viability of zebrafish hepatocytes. ZF-L cell line toxicity represents the first report on the toxicity of a guanitoxin-producing strain in cellular assays with *D. rerio*. The study contributes new information to the scientific literature on the toxicity of a guanitoxin-producing strain in zebrafish hepatocytes, which has shown promise as a biomarker in ecosystems impacted with *S. torques-reginae*. We showed evidence that guanitoxin monitoring is urgently needed and can be part of the government's public health policy. The toxicity responses alert the biological risk to aquatic fauna of an ecosystem contaminated.

#### 1.06.P-Th033 Are Early Life Stages of Fish Affected by Paroxetine? A Case Study with *Danio rerio*

**Carla Ferreira Melo<sup>1</sup>, Cátia Venâncio<sup>2</sup>, Peter Kille<sup>3</sup> and Miguel Oliveira<sup>1</sup>,** (1)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Aveiro, Portugal, (2)University of Coimbra, Portugal, (3)Cardiff University, United Kingdom Paroxetine (PAR) is a selective serotonin re-uptake inhibitor (SSRI) antidepressant that has been increasingly detected in surface waters worldwide raising concern toward potential detrimental effects on nontarget organisms. Therefore, there is the need to understand the potential effects of PAR on fish to increase the knowledge regarding potential environmental impacts. Thus, the present study aimed to assess the short-term effects of PAR (commercial and active ingredient formulation) to early life stages of zebrafish (*Danio rerio*) focusing on lethality, developmental abnormalities, and locomotor behaviour. Organisms were exposed up to 144 h to a concentration range of PAR (40 µg/L to 13500 µg/L) and lethality, morphological abnormalities, as well as swimming activity were assessed. Paroxetine commercial formulation (PAR-c) proved to be more toxic than PAR active ingredient formulation (PAR-a) and the juvenile stage displayed higher sensitivity. Paroxetine induced morphological abnormalities (scoliosis) in a dose-dependent manner, but only from 96 h postfertilization onward, suggesting that PAR toxicity may be related to fully development of the biotransformation system. Moreover, PAR-c exposure (40 µg/L and 400 µg/L) significantly decreased fish swimming activity in both dark and light periods and demonstrated ability to interfere with fish stress response induced by sudden light transitions (dark/light and light/dark). A 21-day period depuration revealed inability to allow full recovery of PAR acute exposed fish. Data highlight the need for more studies regarding effects of PAR to fish.

#### 1.06.P-Th034 The Zebrafish Embryo – An Alternative to Mammalian Teratogenicity Tests in Assessing Effects of Pharmaceuticals?

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Therapeutic drugs represent one of the greatest benefits, but also challenges, to modern society. Via improper disposal, industrial

waste, and wastewater treatment plants, pharmaceuticals are increasingly released into natural ecosystems, potentially expressing effects in nontarget organisms. To protect the environment and the health of humans, hazard and risk assessments of these chemicals became mandatory in the registration process and ecotoxicological studies. However, the rapid increase of toxicity tests, which were mostly performed in mammalian model organisms, led to ethical concern in the scientific community and the public. Thus, there is a need for the development and implementation of alternative methods for toxicity and ecotoxicity testing.

In accordance with this, zebrafish (*Danio rerio*) embryos were investigated for the potential to identify mammalian neuro- and liver toxicity induced by the antiepileptic drug valproic acid and up to 14 analogues. For the identification of neurotoxic potencies, substances were tested with the Fish Embryo Acute Toxicity (FET) test; a subsequent comparison of selected FET endpoints to *in vivo* mouse data expressing exencephaly resulted in a good correlation between zebrafish embryos and mice.

For liver toxicity, histological evaluations revealed drug-induced liver alterations. Additional comparisons of the deduced hepatotoxic potency to the molecular structure of each compound led to a structure-activity relationship, which had, so far, only been documented in yeast cells, mosquito larvae, *Xenopus* embryos, mice, rats, and human HepG2 cells. Drug induced accumulation of lipid droplets as a marker of steatosis, however, could not be documented for valproic acid or any analogue, although toxicokinetic analysis revealed an accumulation of the parent compounds in zebrafish embryos.

In summary, although steatosis as an endpoint in liver toxicity could not be seen with the selected substances, a good correlation between zebrafish and mammals could be observed for valproic acid induced neurotoxic effects and the structure-activity relationships based on hepatotoxic potencies. Considering the variety of therapeutic drugs, zebrafish embryos present a versatile tool in the evaluation of toxic and teratogenic potencies of pharmaceuticals in vertebrates.

#### **1.06.P-Th035 Effects of Amitriptyline and Metabolite Nortriptyline on Eye Development in *Danio rerio***

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Marine ecosystems are exposed to a multitude of different active pharmaceutical ingredients (APIs), of which the nontarget effects on marine biota are not fully understood. The antidepressant amitriptyline has been shown to have the potential to bioaccumulate and to cause long lasting behavioural effects in zebrafish (*Danio rerio*). To better understand the source of these behavioural changes, our study aimed to investigate the effects of amitriptyline, as well as its metabolite nortriptyline, on the eye development of zebrafish larvae. To test the hypothesis that adverse development of the eyes is the source of behavioural changes, a series of endpoints were investigated. Sublethal concentrations of amitriptyline (300, 3.00, and 0.30 µg/L) as well as nortriptyline (300, 3.00 and 0.30 and 0.03 µg/L) were tested and compared against a negative control of culture water, a solvent control of 0.01% DMSO, and a positive control of 3.5 µM phenanthrene. Endpoints include the morphology of the eye at 72 h and 96 h postfertilization (hpf), as well as histology of the eye after 96 hpf. Additionally, expression of genes associated with eye and neuronal development (e.g., *EGR1*, *ptgdsb*, *crybala*) will be assessed after 24, 48, 72, and 96 hpf to better understand how these substances may affect early development of embryos and larvae. Preliminary results show a decrease of eye diameter of 1.2% and 0.72% after 72-h treatment with 300 µg/L amitriptyline and nortriptyline, respectively, which was not found to be statistically significant, and a decrease of 4.6% after exposure to phenanthrene, which was statistically significant ( $\alpha = 0.05$ ). At 96 hpf, a significant decrease in eye diameter was measured again when comparing larvae exposed to phenanthrene to those exposed to DMSO (3.1%), but not for groups exposed to nortriptyline or amitriptyline. The fraction of coagulated eggs ranged from 0% to 10%, with no correlation between lethality and concentration, which confirmed that the exposure scenario was within the sublethal range. Although the tested APIs did not show a statistically significant effect on the eye diameter in zebrafish larvae, histology and gene expression analyses will be carried out as those are known to be highly sensitive endpoints compared to physiology.

#### **1.06.P-Th036 Does Light Pollution Affect Zebrafish Embryos Response to Antineoplastic Drugs?**

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As most of the world's population lives in urban areas, urban aquatic ecosystems are currently subjected to multiple stressors such as light and chemical pollution. Studies dealing with artificial changes in lighting conditions have shown that artificial light at night (ALAN) disrupts biological rhythms and also reduces biological fitness through changes in melatonin levels.

In addition to light pollution, urban aquatic ecosystems are subjected to emissions from hospitals, communities, and industry, increasing concentrations of human pharmaceuticals. As the incidence of cancer increases worldwide, the number of antineoplastic drugs prescribed has also increased exponentially. The immunosuppressant mycophenolic acid (MPA) is one of the most widely used cytostatic drugs due to its successful use in the prevention of rejection of transplanted organs, autoimmune diseases treatment, and angiogenesis inhibition. This drug is considered a priority cytostatic due to its high-volume use, resistance to wastewater treatments, and presence in surface waters (up to 600 ng/L).

Given the ubiquity and co-occurrence of light pollution and antineoplastic drugs, it is critical to evaluate their combined impacts on urban aquatic ecosystems. The mechanistic relationships that determine how changes in lighting conditions affect early life stages of fish and their resistance to antineoplastic agents are currently not understood.

Given the central role played by melatonin, this limitation needs to be addressed. In addition to regulating circadian rhythms, melatonin also regulates antioxidant activity, immune and metabolic and behavioural processes. In this context, this work aims to understand how the exposure to ALAN and the expected change in melatonin levels modulate mycophenolic acid toxicity in zebrafish (*Danio rerio*) embryos. A combination of ecotoxicological approaches were performed with zebrafish embryos, where both ALAN (from light emitting diodes), exogenous melatonin (to counter the expected ALAN-induced reduction of internal melatonin), and sublethal concentrations of mycophenolic acid were manipulated. The parameters analyzed include fish embryo development, heart rate, behaviour, oxidative stress biomarkers, and melatonin levels. The results obtained will allow to what degree ALAN affects zebrafish embryos to be assessed, if exogenous melatonin counteracts ALAN-induced effects, and how ALAN mediates the response of fish to mycophenolic acid exposure.

#### **1.06.P-Th037 Impairment of Sensory Organ Development in Petroleum-Exposed Zebrafish Embryos — Response of the Visual System**

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Crude oil not only serves as an energy source but also forms the foundation for commonly used products such as polymers, chemicals, and pharmaceuticals. Since the demand for these products is rising steadily, also oil exploration and transportation is predicted to increase over the upcoming decades. Consequently, the risk emerging from oil releases to the environment permanently exists. Substances released to the water phase partition rapidly; thus the immediate application of appropriate measures after an oil spill is of high relevance. One of the most abundantly applied methods is the use of chemical dispersants, which are known to increase acute toxicity on exposed aquatic species. Besides the well-examined effects of oil on the cardiovascular system of various fish species, recent studies with zebrafish also found evidence for potential neurotoxicity and, in particular, oculotoxicity. Within this work we address the impact of crude oil and associated dispersants on the visual system in zebrafish and aim to understand underlying mechanisms leading to the observed eye impairment. Zebrafish embryos were exposed to sublethal effect concentrations (EC<sub>10</sub>) of (I) crude oil, (II) dispersed crude oil, and (III) dispersant for up to 120 h postfertilization (hpf). The eyes of exposed zebrafish larvae were examined for structural and functional impairment. The structural analysis was performed using immunohistology of red-green double-cones in cryosections of larval retina. These photoreceptor cells are known to occur most abundantly in the zebrafish retina and facilitate the evaluation of the thickness of the outer and inner segment. Additionally, immunostaining of the less frequently occurring UV-cone provides information about the composition of the retina. For the investigation of the functionality, we used a transgenic zebrafish line that expresses fluorescent lw2-opsin, which is responsible for longwave red-light detection and expected to occur in high numbers in the zebrafish retina. Opsins form a group of proteins facilitating the phototransduction. To further investigate the impact on the developing sensory system, a set of behavioural endpoints was analyzed in 24, 72, 96, and 120 hpf embryos (spontaneous tail coiling, touch-evoked response, light/dark transition test). Results of the structural and functional analysis will support the further characterization of toxicity pathways of crude oil fractions at environmentally relevant concentrations.

#### **1.06.P-Th038 Impairment of Sensory Organ Development in Petroleum-Exposed Zebrafish Embryos – Response of the Lateral Line System**

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Although we are at a turning point in the transformation of the global energy sector, we continue to observe an increasing demand for crude oil in modern society. Hence, through steadily increasing oil production, consumption, transportation, and rapidly moving exploration, huge and small diffuse oil spills still pose a severe risk to the aquatic environment. Representative for the pelagic community, fish early life stages exposed to crude oils, e.g., during spawning season, were found to be highly sensitive. Recent studies provide evidence that in addition to well-known cardiotoxic effects, neurotoxicity also plays a role. In this respect, impairment of development and functionality of the visual system has been shown. Besides eye development, other sensory organs may be affected by oil exposure. In this context, the aim of the present study was to investigate whether water-accommodated fractions (WAF) of native and chemically dispersed crude oil impact the development of the lateral line system (LLS) of fish. This distant-touch sensory system is involved in a multitude of behavioural traits and consequently crucial for survival. We expected a sensitive response to WAF exposure due to the direct contact of LLS hair cells with the surrounding medium. A set of morphological and behavioural endpoints was examined in the transgenic zebrafish line *cldnb:lyn-eGFP*. Expression of a membrane-localized GFP under control of the claudinb promotor visualizes the lateral line primordium and

neuromast cells, facilitating tracing of *in vivo* LLS development. Shortly after fertilization, zebrafish embryos were exposed to different WAFs at sublethal effect concentrations that do not induce visible morphological malformations (EC<sub>10</sub>). Using confocal laser scanning microscopy, LLS assessment in developing embryos (48 hpf-72 hpf) was conducted by analyzing the number of neuromasts along one side of the embryo's trunk. Potential impairment of neuromast structure was quantitatively investigated by DAPI (nuclei)/DASPEI (hair cells) staining. As an endpoint of developmental neurotoxicity spontaneous tail coiling in 24 hpf zebrafish embryos was investigated. First results indicated a delay in neuromast development, a significantly reduced number of neuromasts, and a significantly reduced spontaneous tail coiling in oil-exposed embryos. Overall, the present results will contribute to a deeper understanding of neurotoxic mode of actions of petroleum exposure.

#### **1.06.P-Th039 Automated Counting, Measuring, and Screening of Zebrafish Embryos**

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Zebrafish Embryo Toxicity (ZET) model is increasingly recognised as a highly valuable toxicity testing platform. The ZET assay focuses on the early stages of embryo development and is considered a more humane model compared to adult zebrafish testing. However, these studies often imply the counting and screening of several hundreds of eggs, which is a time-consuming procedure highly subjected to human error. D-Counter (TekenBio) is equipment for automated detection of small organisms (such as daphnids) avoiding laborious manual work, such as counting of organisms. Recently, it has been also tested and validated for counting zebrafish eggs/embryos, a model widely used in ecotoxicology and health sciences. In this work D-Counter was used to study the fecundity of zebrafish couples kept in the University of Aveiro facilities in what concerns number of eggs per batch, viability, and egg size (diameter). Thus, D-Counter was used for i) counting and measuring 2.5 h postfertilization eggs of each batch, and ii) counting discriminately the number of coagulated, nonviable (unfertilized or with abnormal development) and viable eggs in each batch. These parameters were also determined manually using a stereomicroscope and compared to the automated measurements to assess the equipment's accuracy. Results indicate a very good correlation between the equipment and the manual measurements ( $r^2 > 0.98$ ). These data will be used to study the correlation between the number of eggs in the batch, embryo size, and embryo viability.

#### **1.06.P-Th040 Answering Environmental Toxicology Questions Through LC-TIMS-HRMS Based Toxicometabolomics of Zebrafish Exposed to Xenobiotics – Lipid Metabolism Under the Spotlight**

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Given the large number of xenobiotics, there is an important gap in the literature concerning their adverse effects on aquatic organisms. The impact of xenobiotics in the aquatic ecosystem is evaluated in more depth when the whole metabolome (xenobiotics, their biotransformation products, and endogenous metabolites/lipids) of aquatic organisms is studied. However, there is a clear need in the literature for further information on biochemical perturbations underlying the mode of action of xenobiotics.

Through toxicometabolomics, a broad range of toxic responses can be captured in an untargeted way. Therefore, toxicometabolomic studies involving zebrafish larvae (ZLF) have become increasingly popular for linking apical endpoints to biochemical perturbations (as part of adverse outcome pathways [AOPs] determination). Lipids are important for many biological functions of a living organism such as energy storage and cell signalling and growth, among others, while they constitute the building blocks of membranes. A number of environmental contaminants have been reported to interact with lipid metabolic pathways and can lead to oxidative stress and lipid peroxidation.

Here, we highlight a liquid chromatography-trapped ion mobility-high resolution mass spectrometry (LC-TIMS-HRMS)-based untargeted toxicometabolomics workflow that i) measures the full complement of detectable endogenous chemicals in tissue samples from ZFE exposure experiments, ii) points out the altered metabolites/lipids, and iii) provides annotations of high confidence. Exposure of ZFL to triclosan (TCS) is used as an example.

Even though the exposure concentration used in the toxicokinetic experiment did not induce any observable effects, significant changes in the lipidome level were reported. Most of the detected lipids were up-regulated while LPC and PC were the lipid classes with the highest signal increase in TCS exposed samples. An overtime increase of the detected lipids was observed, demonstrating lipid accumulation as a toxicity effect of TCS. These observations can be linked with inhibition of beta-oxidation, peroxisomal stress, and hepatic lipotoxicity. Overall, the considerable breadth and depth of detectable lipids are encouraging for using ZFL and untargeted MS-based lipidomics to probe a variety of responses to environmental exposures as well as for evaluating impacts from individual high priority chemicals. Moreover, the developed toxicometabolomics workflow captures a broad range of toxic responses in an untargeted fashion that would meet the needs of modern environmental toxicology.

#### **1.06.P-Th041 Use of Zebrafish Embryotoxicity and Reprotoxicity Tests to Assess Environmental Chemicals Effect on Reproduction and Development**

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The ICH S5 (R3) guideline on reproductive toxicology that applies to all pharmaceuticals for which reproductive and/or developmental toxicity studies are appropriate proposes the use of alternative assays as part of an integrated testing strategy to minimize the use of animals. The guide provides a list that contains 29 Reference chemicals that have been shown to induce specific malformation or embryo-fetal lethality plus three Negative Chemicals that can be used to support the sensitivity of an alternative assay.

Our research focused on the predictivity of the zebrafish developmental and reproductive toxicity assays. Zebrafish is a highly popular model in environmental toxicology and provides an ethically acceptable small-scale analysis system with the complexity of a complete organism. This model enables continuous developmental monitoring as well as simple adult testing and has been widely used for the generation of relevant answers on mammalian developmental and reproductive risk assessment and hazard. Our goal is to further validate this model for its use by testing the compounds indicated in the ICH S5 guideline and complementing them with other endpoints. To determine the teratogenic risk of these chemicals, the presence of morphological alterations in zebrafish embryos is analyzed at two different stages and the teratogenic indexes (TI) are established as the ratio between LC50/EC50 for each stage. As this assay is performed in externally fertilized embryos, embryotoxicity is directly analyzed, but reprotoxicity could be studied further evaluating the effect of some of the listed compounds in adult zebrafish males by the *in vivo* sperm quality assessment. In this assay, the development and maturation of gametes is evaluated by assessing the sperm quality (progressive motility, curvilinear velocity, and linearity) by CASA analysis and even expression of specific marker genes (ziwi, sycp3l, and shippo1) by qPCR.

This assay complements the developmental toxicity with the effect on the male reproductive system, having a more accurate evaluation of embryotoxicity and reprotoxicity in the same model, helping to increase the accuracy and sensitivity of the teratogenicity assay by more than 80% and 88%, respectively, compared to mammals; therefore, this is a well-integrated strategy to minimize the use of mammals to predict developmental and reproductive toxicity effects, both in the zebrafish model.

#### **1.06.P-Th042 Offshore Renewable Energy: Toxicity of Metallic Elements Released in the Marine Environment by Anticorrosive Protections in Medaka (*Oryzias melastigma*)**

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Cathodic protections such as galvanic anodes (GACPs) are used to prevent corrosion of metallic materials that are immersed in seawater. The exponential development of offshore windmill parks raised questions regarding toxicity of GACP-released metallic elements toward marine organisms. In this study, we exposed medaka (*Oryzias melastigma*) as a model species to (i) the cocktail of elements released from a functioning aluminum-based galvanic anode, or (ii) a solution of aluminum chloride used as a reference. Fish were exposed during their entire life cycle (fertilization up to three month adults). Seven dilutions were prepared to cover a wide range of concentrations. To evaluate toxicity, the following endpoints were monitored: survival, growth, and reproductive output. In addition, several behavioural endpoints were evaluated to assess potential negative effects on exploratory abilities, learning (associative and memory), spontaneous activity, and anxiety. Toxicity key values such as LC50, EC50, No Observed Effect Concentration (NOEC), and Lowest Observed Effect Concentration (LOEC) were calculated for chemical risk evaluation.

Monitoring of elements released by the galvanic anode revealed that fish were exposed to aluminum total concentrations ranging from ~2000 µg L<sup>-1</sup> down to ~70 µg L<sup>-1</sup>. Preliminary results show no acute toxicity (survival, teratogenicity, locomotion) at concentrations ≤ 2000 µg/L of total aluminum on early-life stages of medaka (up to 12 d postfertilization). Long-term exposures are still ongoing and the data (available in the beginning of 2023) will be used to refine the chemical risk analysis for aluminum and to define it for GACPs. Overall, the results of this study will serve as a basis to give recommendations to the different stakeholders on the safe use of cathodic protections in future projects related in particular to the development of offshore renewable energy.

#### **1.06.P-Th044 Barrier Function of the Marine Medaka (*Oryzias melastigma*) Chorion**

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The marine medaka, *Oryzias melastigma*, has been identified as a promising model species for marine ecotoxicology and shares many advantages for early life stage testing with the well-established freshwater model species zebrafish (*Danio rerio*). Therefore, the standardized Fish Embryo Acute Toxicity (FET) test has been successfully adapted to *O. melastigma* within previous work. To allow a comparison to other model species, different species-specific aspects such as development, metabolism, and sensitivity have to be considered. In this context, the present study focused on the chorion barrier function. The protective role of the chorion as a physical barrier has already been identified as an important factor for the uptake of chemicals and thus the resulting toxicity. As the chorion of medaka is about 10x thicker compared to zebrafish, this might be an indication for an increased barrier function resulting in deviating uptake kinetics. In a first experiment the influence of solvents (DMSO) on

the chorion permeability and thus, the compound uptake, was investigated in *O. melastigma* and *D. rerio* using fluorescent dyes. Our results confirmed previous findings that solvents increase the chemical uptake and supported the hypothesis that the medaka chorion provides a stronger barrier compared to zebrafish. Furthermore, structural properties of the chorion were analyzed using scanning and transmission electron microscopy (SEM, TEM) with particular interest on the property of the pore canals and the overall chorion surface conditions after the commonly applied removal of villi as a pretreatment step within FET. In this context, also the molecular weight as a relevant criterion for chorion passage was evaluated. To identify the molecular weight threshold for the transport of compounds through the pore canals, medaka embryos were exposed to solutions of polyethylene glycols (PEG) with different molecular weights. Solutions were prepared in hyperosmotic concentrations, which caused an immediate loss of fluid and shrinkage of the chorion. Eggs exposed to PEG solution containing glycols < 6000 kDa were able to regain the original shape and equilibrium by uptake of surrounding solution. Hence, the higher molecular weight PEG solutions were most likely not able to pass the chorion. Additionally, an enzymatic dechoriation procedure was optimized to further investigate the role of the chorion and to extend the potential for early life stage testing with the marine medaka.

#### **1.06.P-Th045 Element Accumulation in the Evil-Eye Blaasop, *Amblyrhynchotes honckenii* (Bloch), and its Parasitic Isopod, *Cinusa tetradontis* Schjödte et Meinert, 1884, in South Africa**

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Marine Protected Areas (MPAs) conserve fish species and promote the conservation of marine life; however, almost nothing is known regarding potential impacts on conserving marine fish parasites. Despite their importance as key components in ecosystems, marine parasites remain severely understudied. Yet, these organisms can successfully be used as bioindicators to determine the success of conservation interventions, as well as the extent of chemical pollution within specific marine ecoregions. A relatively novel field within ecotoxicology is environmental parasitology, a field that deals with the interaction between parasites, their hosts, and environmental pollution. Recognizing the need for a more interdisciplinary research approach, this study combines parasitology with ecotoxicology to determine the potential of the parasitic isopod, *Cinusa tetradontis* Schjödte et Meinert, 1884, of the Evil-eye pufferfish, *Amblyrhynchotes honckenii* (Bloch), as a bioindicator for element accumulation both inside and outside MPAs. The concentrations of Al, As, Cd, Cr, Cu, Mn, Hg, Ni, Pb, Se, and Zn were determined in muscle and liver tissues of both infected and uninfected fish, as well as parasites from Witsand, in close proximity to an MPA, and Mossel Bay, outside an MPA, on the South African temperate coast. Concentrations of Cu, Mn, Ni, Pb, and Zn in *C. tetradontis* differed significantly between the two sites, where Mossel Bay (outside an MPA) had higher concentrations of Ni, Pb, and Zn. Interestingly, Hg concentrations were higher in samples from Witsand compared to Mossel Bay, with the exception of uninfected liver tissues. The parasitic isopod, *C. tetradontis*, accumulated significantly higher concentrations of Al, As, Cu, Ni, Pb, Se, and Zn compared to its fish host. In general, infected fish had lower concentrations compared to uninfected fish. Only Cd levels in liver tissues of uninfected fish in Mossel Bay were significantly lower than the levels in infected fish. This study highlights the importance of utilizing this interdisciplinary approach, and the benefits of utilizing parasitic bioindicator species in the management of MPAs and future conservation strategies.

#### **1.06.P-Th046 Hepatic Morphological Acute Damages of Neotropical Fish Exposed to Low Concentrations of Fire Suppression Agents (FSA)**

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Fire Suppression Agents (FSA) are used worldwide in firefighting in general. They form a foam covering, thus providing a physical impediment of oxygen and isolating the flammable surface from contact with fire. They also act by cooling the surface of the material, such as the flame retardants. In 2015, fuel tanks of the Petrochemical Terminal of the Santos Harbor caught fire and thousands of FSAs were used and reached the Santos estuary. Therefore, this study aimed to evaluate the histopathological effects of different FSAs in hepatic tissue of neotropical fish. For this purpose, three brands of FSAs were tested: Ageo (A), Cold Fire (C), and F500 Fire (F). The *Poecilia reticulata* (guppy, animal model) were acclimatized and maintained at 25°C±2 °C, under constant aeration with photoperiod of 12h:12h light:dark and salinity of 14. They were fed daily *ad libitum* with Alcon Basic®. Twelve specimens were used for different brands and concentrations. The groups were: control (CTL), concentrations 0.1% (I), 0.001% (II), and 0.00001% (III) in duplicate in 5 L aquariums for each treatment for 96 h. Then, the animals were euthanized, the livers were collected, and they were submitted for the histological routine; 5 µm cuts were stained with hematoxylin-eosin (HE) and Masson's Trichrome (MT). The concentration I was lethal in all treatments, making the analysis unfeasible. Survival occurred at concentrations II and III only for Ageo, and for Cold Fire and F500 Fire at the lowest concentration (III). The CTL group livers showed normal parenchymal morphology. In the treatment with Ageo, the livers showed hypertrophy of blood vessels and venous congestion, in addition to sparse vacuolation of hepatocytes. At the lowest concentration, both the Cold Fire and F500 Fire brands showed hemorrhage and a few lymphocytes were found associated with the hemorrhage centers and the vessel branches in the parenchyma. In addition there was necrosis associated with blood vessels. The predominance of vacuolated cells throughout the hepatic parenchyma, filled with mainly water, indicated acute edema caused by the chemical lesions by the FSAs, resulting in necrosis. We concluded that all FSAs used in the test led to acute morphological responses in all concentrations, without any inflammatory response. The lethality was at morphological level with any possibility of biochemical, immune, and physiological responses, as already described in previous studies by the other authors.

## 1.06.V Fish Model Species in Human and Environmental Toxicology

### 1.06.V-01 Neurotoxic Effects by Bifenthrin Exposure in Zebrafish

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Bifenthrin, a pyrethroid insecticide, is used to treat various crops. Bifenthrin consequently has the potential to disrupt both terrestrial and aquatic populations since its effects are propagated across invertebrate life stages and generations, trophic levels in aquatic food webs, and ecological barriers to riparian food webs. In spite of this, studies of bifenthrin in aquatic organisms are lacking.

Wildtype (WT) and transgenic (TG) [*Tg(elavl3:EGFP)* and *Tg(mbp:mGFP)*] zebrafish were exposed to 0, 0.1, 1, 11.1, 33.3, 100, and 300 µg/L for 120 hpf. Phenotypic assessments including hatching rates, survival, normal development, tail coiling and touch evoked responses, body length, and weights were undertaken from 24 hpf. Furthermore, at 120 hpf brightfield images were taken for the WT zebrafish, and fluorescence imaging was done for the TG lines. Genes related to autism and developmental neurotoxicity were also screened. Zebrafish at 120 hpf was also monitored to track its locomotor activity.

After 72 hpf, the 100 µg/L and 300 µg/L exposure group started showing signs of abnormal development, and this was evident in their body lengths, too. Moving duration, distance moved, body contact, and proximity were all significantly affected by bifenthrin exposure. Also, three genes related to autism were significantly affected; nine genes screened for developmental neurotoxicity were all significantly affected. Neurogenesis and myelination processes were disrupted as seen through fluorescence imaging with the TG lines. Both phenotypically and genotypically, zebrafish systems are significantly distressed by bifenthrin.

From the results obtained so far, zebrafish establishes itself as an excellent alternative model for neurotoxicity testing, hereafter, bifenthrin's neurotoxicity on zebrafish is found.

### 1.06.V-02 Development of a Toxicity Fingerprinting System to Identify Developmental and Acute Neurotoxicants in Larval Zebrafish

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Exposure to an increasing number of environmental chemicals has been associated with acute neurotoxicity (ANT) or developmental neurotoxicity (DNT) in humans. The majority of chemicals in commerce have not been evaluated for ANT/DNT because of a lack of next-generation toxicity tests. We developed an 11-endpoint behavioural battery to assess ANT/DNT in larval zebrafish. This new approach method (NAM) contains endpoints for the light-dark transition test, acoustic and visual startle responses, habituation learning and potentiation, and memory retention. This work describes the ongoing development of a toxicity fingerprinting system that can be used to identify potential underlying mechanisms by which exposure to environmental chemicals causes ANT/DNT. To build acute neurotoxicity fingerprints, 5-d postfertilization (dpf) zebrafish were exposed to compounds (0.8 µM–120 µM) that target neurodevelopmental receptors including the GABA-B (CGP13501), NO-cGMP (ODQ), and NMDA (MK-801) receptors for 60 min before behaviour was assessed. Acute behavioural fingerprints for the toxicologically relevant receptors PPARα (GW7647) and PPARγ (T0070907) were generated. Compounds previously described to be nontoxic to the developing nervous system in human *in vitro* assays were also assessed (fluconazole, sodium benzoate, sodium saccharine hydrate) and all fingerprints were generated relative to the vehicle control (0.4% DMSO). In line with human *in vitro* DNT data, acute exposure to fluconazole, sodium benzoate, or sodium saccharine hydrate did not alter swimming behaviour. These compounds were classified as negative control chemicals for the zebrafish ANT assay. Exposure to the remaining chemicals produced a wide array of toxicity fingerprints comprised of chemicals that caused decrements in habituation learning (MK-801) or changes in the visual (GW7647, CGP13501, T0070907, ODQ) and/or acoustic startle (GW7647, CP-673451, ODQ, CGP13501, T0070907) response, with varying directionality. Future work will expand the acute fingerprinting system to cover additional neurodevelopmental and toxicological pathways. In summary, this work deployed a novel zebrafish NAM to generate acute toxicity fingerprints. In the future, the same strategy will be applied to generate DNT fingerprints. Ultimately, the ANT/DNT fingerprinting system will be deployed to identify potential mechanisms by which environmental chemicals cause neurotoxicity outcomes using a 3R compliant, alternative test system.

## 1.07.P Freshwater and Marine Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms

### 1.07.P-Th047 Raman Spectroscopy and Structural Analysis of Cyanobacteria and Harmful Algal Blooms

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Cyanobacteria, also known as blue-green algae, represent the majority of harmful algal blooms which can appear in marine or fresh water. The presence of such algal blooms is a problem for bathing lakes as well as drinking water or food production in general. The reason for this is that the cyanobacteria are capable of producing cyanotoxins which can be harmful and potentially even lethal to humans, animals, and aquatic life. In principle, by simple visual inspection of the bloom, it cannot be determined whether an algal bloom is harmful to the environment or not. Currently, to assess the type and toxicity level of algal blooms



laboratory-based analytics are used which is time consuming and also cost intensive. Thus, fast and ideally real-time as well as reliable and highly sensitive concepts for the analysis of algal bloom in aqueous environment are required.

Generally, Raman spectroscopy, also known as fingerprint spectroscopy, is capable of analysing the structure of algae and bacteria noninvasively, label-free, and without physical contact. The technique delivers molecular information of the content of biological samples and, therefore, enables a fast differentiation and determination of algal or bacterial cells.

In this work, we present a concept based on Raman spectroscopy and imaging to study, analyse, and quantify harmful or toxic cyanobacteria or algae from liquid samples. To demonstrate the capability of our approach, we investigate different species of cyanobacteria and show that on the basis of the acquired Raman spectra, a detection of harmful algae and cyanobacteria is possible. Raman measurements are performed in the visible wavelength range using an in-house measurement setup. To obtain structural information from the inside of the cells, the cells need to be bleached prior to the analysis and processing of the Raman spectra.

Consequently, processed Raman spectra, recorded from such samples, deliver information about the structural components and also about the cell composition.

We will present the experimental Raman setup developed for the studies and show our latest results on the reliable assessment of harmful cyanobacteria and algae. Also, we discuss next steps to automated real-time measurements and monitoring.

#### **1.07.P-Th048 Monitoring Cyanobacteria Levels in Recreational Waters: Success with STEM in a Community Sailing Program**

**Dana Norton<sup>1</sup>, Gary C du Moulin<sup>2</sup>, Leonardo Parodi Ambrose<sup>3</sup>, Connor Quigley<sup>1</sup>, Anna Lena Leutiger<sup>1</sup>, Charles Zechel<sup>1</sup> and Yakira M Becker<sup>4</sup>,** (1)Community Boating Inc., Boston, (2)Quality Systems and Compliance Strategies, (3)Boston University, (4)Northeastern University Harmful algal blooms (HABs) are becoming more common in urban waterways due to human influences. Harmful algal blooms are elevated levels of algae and bacteria in an aquatic environment and can negatively affect local ecosystems and human health. Cyanobacteria is photosynthetic bacteria that releases toxins called cyanotoxins that are harmful to human health and a common species for HABs in the lower basin of the Charles River in Boston, MA, USA. Community Boating (CBI) offers direct access to the Charles River for thousands of people, increasing potential for exposure to cyanobacteria HABs. This research aims to track development and dynamics of cyanobacteria population within CBIs operational area, assist government agencies in tracking trends due to climate change and current land use practices, and assess waterbody/human health vulnerability. A graduate toxicology student and a professional microbiologist oversaw junior sailors to teach scientific methodologies including sample collection and microscopic analysis for this project. Samples were collected in two locations: Longfellow Bridge area (depth ~33 feet), where most sailing activities occur, and the Mooring field (depth ~6 feet), where watercraft are launched. Each site was sampled with a 3-meter-long plankton net pull with a 50- $\mu$ m mesh net and a 3-meter-deep integrated tube sample.

Approximately 200 samples were collected at consistent times each day by boat. Samples were processed using fluorometry and microscopy to determine cyanobacteria density and speciation. Surface water temperature, air temperature, GPS coordinates, and time were recorded for each sample. Phycocyanin, which represents the presence of cyanobacteria, was found to be between 25 mg/L and 100 mg/L at the Longfellow Bridge and 50 mg/L to 100 mg/L at the Mooring field. There were no HABs reported during the time of testing. This study happened in severe drought conditions; therefore runoff and combined sewer overflows (CSOs) had little or no impact in contributing pollutants. The lack of CSOs during this summer's investigations provided insights into the natural presence of cyanobacteria without the complicating component of excess pollution. Cyanobacteria bioconcentration in the Charles peaks in July as a potential result of longer daylight hours and increase in nutrients. This study provides valuable input into further investigations that can provide guidance to stewards of the river environment as well as enhance the safety of river recreation.

#### **1.07.P-Th049 Development of a *Microcystis aeruginosa* Culture Method to Produce Sufficient Amounts of Microcystin to Conduct Multispecies Acute and Chronic Toxicity Tests**

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There is a lack of information to estimate safe exposure levels of toxins produced by cyanobacteria to freshwater aquatic life. The uncertainty in concentrations and purity of standards for cyanotoxins and their costs present a challenge for conducting acute and chronic toxicity tests. To address this, different approaches to culture cyanobacteria for toxicity assessments were compared. Microcystin-producing *Microcystis aeruginosa* (with *mcy* genes confirmed by qPCR and RT-qPCR) was used in these cultures. Toxins were collected by removing cells from culture media by centrifugation and then lysing the cells by freeze thawing them three times. Initial culture efforts using 2-L to 80-L cultures grown for similar periods (~150 days) in BG11 media resulted in a range of cell densities (3.7-4.4 x 10<sup>6</sup> cells/mL) and consequently a range in total microcystin concentrations (37-73  $\mu$ g/L) of the lysates. Another method using a different culture volume (eight flasks of different ages combined into 21 L), similar age of culture (~150 days), and a cell density of 1.7 x 10<sup>6</sup> cells/mL, resulted in a microcystin concentration of 885  $\mu$ g/L. This culture volume was repeated and resulted in a cell density of 1.4 x 10<sup>6</sup> cells/mL and a microcystin lysate concentration of 426  $\mu$ g/L. Due

to the apparent variability in toxin yields, an approach was taken to grow *M. aeruginosa* cultures specifically up to a stationary phase in 250-mL and 1-L flasks. However, final toxin concentrations from the cell lysates were too low to be suitable for adequate aquatic toxicity testing, with microcystin concentrations of only ca. 25 µg/L. A final method developed using an aerated culture under nutrient replete conditions with lower temperature and illumination in two carboys containing 14 L culture media in each, resulted in a 28-day cell density of  $9.5 \times 10^6$  cells/mL and a concentrated lysate microcystin concentration of 9400 µg/L. This culture method was repeated two additional times and resulted in cell densities of  $2.6 \times 10^7$  cells/mL (28 days) and  $2.5 \times 10^7$  cells/mL and  $2.3 \times 10^7$  cells/mL (21 days). Large batch culture acute toxicity tests resulted in LC50s of 528 µg/L for *Ceriodaphnia dubia*, 892.6 µg/L for *Daphnia magna*, and 892.6 µg/L for *Neocloeon triangulifer*. For chronic tests, IC25s for the small batch cultures for total microcystins for *C. dubia*, *Pimphales promelas*, *Hyalella azteca*, and *N. triangulifer* were 8.9, 74.0, 408.9, and 10.1 µg/L, respectively. Additional results will be presented.

#### 1.07.P-Th050 Effects of Binary Mixtures of Cyanotoxins and Xenobiotics on the Growth Rate of the Freshwater Algae *Chlorella vulgaris*

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Aquatic organisms are constantly exposed to several natural and anthropogenic contaminants such as cyanotoxins, metals, and pesticides in eutrophic brackish and freshwaters mainly due to toxic cyanobacterial blooms and human activities. However, the environmental risk of their combinations with the aquatic environment is still unknown. The present study aimed to investigate the growth response of *Chlorella vulgaris* exposed for seven days to four single-chemical compounds (microcystin-LR, cylindrospermopsin, terbuthylazine, and cadmium) and five binary mixtures. In the single-chemical exposures, terbuthylazine was the most toxic to *C. vulgaris*, followed by cadmium, cylindrospermopsin, and microcystin-LR, which was the least toxic. The MIXTOX tool was used to compare the obtained data from the laboratory experiments with the predicted data generated by the conceptual model for independent action based on individual toxicities. Deviations for synergistic/antagonistic interactions, dose ratio, and dose level dependency were also tested for the best data fit. Several response patterns were obtained in the mixture exposures depending on the mixture. Antagonism was the prevailing interaction between the chemical compounds in a 4-d exposure period, while dose level dependency was the only deviation obtained in a 7-d exposure period. In the case of dose level dependency, synergism at low doses of each chemical compound was observed in three of the five tested binary mixtures. This synergistic effect represents a significant risk for aquatic organisms simultaneously exposed to cyanotoxins and anthropogenic contaminants in the environment.

#### 1.07.P-Th051 Nutrient Limitations and Differential Gene Expression of *Prymnesium parvum* Throughout Growth Curve

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Anthropogenic activities are known to imbalance the nutrient cycle in environmental systems. *Prymnesium parvum* is an algal species known to produce harmful algal blooms that are responsible for numerous fish kill events worldwide. Some studies have advanced our knowledge of *P. parvum* toxin production; however, the genetic underpinnings of toxin production still need clarification. This study aims to provide a detailed transcriptomic analysis of *P. parvum* throughout its growth phase during natural conditions and during pesticide exposures to determine if pesticides can alleviate stress-related responses, as suggested by prior studies. The hypotheses for this study are (1) expression of genes required for *Prymnesin* synthesis will increase throughout *P. parvum* growth phase as toxicity increases, (2) decreased nitrogen and/or phosphorus increases *P. parvum* toxicity, and (3) glyphosate (and its metabolite AMPA) help compensate for nitrogen and phosphorus depletion. For this investigation, *Prymnesium parvum* cultures were maintained in a modified UTEX artificial seawater medium [ASM]. RNA was extracted from three replicates at three different growth phases, analysed for integrity, and sequenced. Nutrient levels were recorded throughout the growth phase, and separate cultures maintaining a constant balance were used for further analysis. To determine toxicity of the *P. parvum* culture, *Daphnia pulex* were exposed to *P. parvum* at concentrations typically found during toxic blooms. Modified UTEX ASM was spiked with environmentally relevant concentrations of glyphosate and AMPA, and RNA extractions and toxicity tests were followed as previously described. Results of toxicity tests from *Daphnia pulex* exposed to *P. parvum* showed increased toxicity throughout the growth curve, with average mortalities of 75% (early exponential), 95% (late exponential), and 100% (late stationary). Preliminary results of RNA sequencing showed differences in gene expression across the growth curve. This research will advance our knowledge of *P. parvum* toxicity mechanisms, thus improving detection and management strategies to reduce toxic bloom frequency and intensity.

#### 1.07.P-Th052 Widely Used Herbicide Promotes Toxigenic Cyanobacterial Growth and Decreases the Reproductive Output of its Parasitic Fungi

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Metolachlor (MET) is a widely used herbicide. It negatively affects phytoplanktonic nontarget organisms, such as cyanobacteria, by inhibiting their growth and photosynthetic activity.

Chytrids are zoospore fungi ubiquitous in aquatic environments that parasitize cyanobacteria and consequently keep their growth in check. To date, little is known about the effect of pollutants on chytrids infections and, consequently, about the ecological processes associated to these parasite interactions. Traditionally, ecotoxicological tests have focused on the single-species level. However, it is pivotal to understand how pollutants affect ecological interactions between species, like host-parasite interactions. Therefore, we tested the effect of environmentally relevant concentrations of MET on the interaction between the toxigenic cyanobacterium *Planktothrix agardhii* and its chytrid parasite *Rhizophydium megarrhizum*.

Cyanobacteria growth was higher in the uninfected treatment at 10 µg L<sup>-1</sup> and 100 µg L<sup>-1</sup> MET than in the control. Thus, MET might promote cyanobacterial bloom formation. Prevalence and intensity of infection were not affected by MET exposure, suggesting chytrids could control cyanobacterial growth promoted by pollutants. However, after 7 d of exposure to the highest MET concentrations, chytrid sporangia were smaller, which may imply a negative effect on the chytrid at longer exposure times. This study shed light on how widely used anthropogenic pollutants could favour cyanobacterial bloom formation. Moreover, it suggests that parasitic chytrid infections might help to control cyanobacterial growth even in polluted environments.

**1.07.P-Th053 Response of a Coastal Amphipod to Single and Binary Exposures of a Toxic Seaweed and a Dinoflagellate**  
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Global environmental changes have been associated with the proliferation of potentially harmful algae species (HABs) in coastal ecosystems, which may release high concentrations of toxic compounds, which can be harmful to inhabiting biota and present ecological consequences to the aquatic ecosystem. *Asparagopsis armata* is a bloom-forming red seaweed that produces an array of organohalogenes, with considerable toxic potential to other aquatic organisms. *Ostreopsis cf. siamensis* is a potentially harmful dinoflagellate for which very little is known about its toxins and toxicity. This study aimed to evaluate the ecotoxicological influence of these two distinct benthic species in the representative invertebrate of coastal environments, *Echinogammarus marinus*. To this end, the amphipod juveniles were exposed to a gradient of macroalgae exudate dilutions (0 to 16%) and live microalgae densities (0 to 14,566 cells.mL<sup>-1</sup>) to evaluate survival after acute exposure (48 h). LC50 values were estimated at 5.84% exudate and 452 microalgae cells.mL<sup>-1</sup>, respectively. Then *E. marinus* juveniles were exposed to sublethal (LC10) concentrations of the stressors, isolated, and mixed, using a simple experimental design. After exposure (48 h), organisms were left to recover during 24 h in a clean medium and were transferred to a medium with *Fucus* sp. Seaweed exudate (food stimulus), and their motility was tracked during 30 s. Motility in amphipods was more affected by *O. cf. siamensis* LC10 than the equitoxic concentration of the exudate from *A. armata*, with an average total distance of about 3.6 times less than the exudate, and 6.8 times less comparatively with control treatment. Exploration rates and frozen events (alive but immobilized) followed the same trend. Combining equitoxic levels of stressors in the same exposure resulted in the same motility effects as those observed for the isolated exudate. Moreover, combining *O. cf. siamensis* LC20 and *A. armata* LC10 resulted in increased motility, almost reaching the observed in the control treatment. Overall, the present work came to demonstrate that both algae species are toxic to other organisms, but also suggests that, depending on their densities in the environment, *A. armata* and *O. cf. siamensis* may neutralize each others' toxic effects in the environment.

**1.07.P-Th054 Toxicity of *Ostreopsis cf. siamensis* and *Prorocentrum lima*: Effects in the Coastal Amphipod *Echinogammarus marinus***

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Benthic harmful algal blooms (BHABs) have been associated with negative impacts in coastal waters, primarily through the production of toxins that affect marine communities and the trophic web. The benthic dinoflagellates *Ostreopsis cf. siamensis* and *Prorocentrum lima*, which occur in temperate marine waters and may give rise to BHABs, have been studied in recent years. However, gaps persist regarding the underlying mechanisms of BHAB toxicity on many invertebrates of estuaries and marine coastal areas. This work aims to evaluate the lethal and sublethal effects of the harmful dinoflagellates *O. cf. siamensis* and *P. lima* on juveniles of the amphipod *Echinogammarus marinus*, and infer about possible pathways of toxicity in this macroinvertebrate. Juveniles were exposed during 48 h to a range of microalgal densities, and acute toxicity thresholds were estimated for survival and immobilization. The ability of recovery from sublethal exposures was assessed after a 24-h period in a clean medium, and endpoints of motility were measured (average speed and total distance). Mechanisms of toxicity were analysed with biochemical biomarkers of neurotransmission (AChE), detoxification response (GST), oxidative damage (LPO), and energy metabolism (ETS and LDH/IDH). A decrease in survival of *E. marinus* juveniles occurred with increasing microalgal densities, comparing with the controls (0 cells/mL). Effects were more pronounced with *O. cf. siamensis* for both 24 h and 48 h (LC50 = 6520 and 2143 cells/mL, respectively) than with *P. lima*, for which LC50 values were estimated to be > 241 800 cells/mL. After 24 h, juveniles exposed to *O. cf. siamensis* were less responsive to stimulus than those in contact with *P. lima* (EC50 = 1844 vs. > 241 800 cells/mL). This trend continued after 48 h (EC50 = 664 vs. 111 851 cells/mL). After exposure at sublethal dinoflagellate densities, juveniles did not fully recover their motility: the average speed registered was significantly lower than in control treatments. These observations suggest a more prolonged toxic effect. In view of these results, and based on preliminary analyses, changes at the biochemical level may occur, particularly in the energy metabolic pathway and in mechanisms of the cholinergic

system. Overall, this work puts in evidence the noxious effects of harmful microalgae species on the gammarid here tested, which may translate into potential consequences for the coastal trophic web dynamics.

#### **1.07.P-Th055 Adaptation Responses of the Cyanobacteria *Halomicronema metazoicum* to Consequences of Climate Change Scenarios**

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Marine communities are nowadays exposed to multiple stressors and to rapid changes in their natural environment. Altogether, such stressors could impact single taxa up to populations with consequences on biodiversity and ecosystem functions. Ocean acidification (OA) is considered one of the major threats of climate changes and may have awful consequences on marine life. The rise in carbon dioxide ocean absorption alters the seawater chemistry leading to surface pH fall and influencing the chemistry of toxic compounds with consequences that have been overlooked. Despite the seriousness of such changes certain species have been found to be able to deal with and tolerate naturally acidified conditions in their natural habitats. Understanding the mechanisms at the base of this resistance could provide useful insight on how marine organisms will respond to multiple stressors in a climate changing scenario. Recent studies have underlined the implication of ATP-binding cassette (ABC) transporters in acid stress response of some Cyanobacteria strains. Here we investigated the potential involvement of ABC in the resistance to low seawater pH (range 7.7-6.5) in the marine cyanobacterium *Halomicronema metazoicum* collected from a natural acidified marine area (Ischia Island, Italy). *Halomicronema* mattes have been exposed to low-pH conditions simulating OA scenarios (7.7, 7.2, 6.5) for 30 days and the expression of ABC-like gene *slr2019* at two time points (7 d and 30 d) have been assessed. All mattes were alive after 7 d and 30 d of exposure and an upregulation of *slr2019* gene has been found at pH 7.7 after 7 d while no gene modulation has been observed at lower pHs (7.2 and 6.5). An hormesis response to decreasing pH values could be hypothesized, being characterized by a rise at lower doses and a decrease with increasing low pH conditions. Such a trend suggests the involvement of ABC response to slightly low pH conditions but possibly being ineffective at lower pHs. Preliminary results show that *H. metazoicum* is able to tolerate low-pH environments and the potential involvement of ABC proteins in acid stress resistance. More research investigating cellular and molecular mechanisms behind adaptive responses to OA could help to predict the consequences of future predicted changes on marine communities and the response of natural population to multiple stressors scenarios.

#### **1.07.P-Th056 Control of Harmful Algae by Infochemicals Derived from *Daphnia* According to Water Temperature and *Daphnia* Species**

**Gwiwoong Nam**, Gersan An and Jinho Jung, Korea University, Korea, Republic of (South)

Harmful algal blooms (cyanobacterial blooms) have been increased due to eutrophication and global warming. Cyanobacteria can negatively affect the environment by releasing toxins such as microcystins causing serious toxic problems. As primary feeders of cyanobacteria, *Daphnia* can control cyanobacteria by direct control such as feeding and indirect control such as release of infochemicals. In this study, we focused on the *Daphnia*-derived infochemical and investigated the control of cyanobacteria using *Daphnia*-derived infochemical. Using infochemicals from *Daphnia magna* (international standard species) and *Daphnia galeata* (domestic species), experiments were performed on *Microcystis aeruginosa* ( $1.00 \times 10^6$  cell mL<sup>-1</sup>). Colony formation, growth rate, reactive oxidative stress (ROS), extracellular, and intracellular microcystin-LR of *Microcystis aeruginosa* were observed over 15 days of exposure. *Microcystis aeruginosa* control effects were confirmed in both species, but *Daphnia galeata* infochemical showed better control ability than *Daphnia magna* infochemical despite its small body volume.

#### **1.07.P-Th057 Graphene-Mediated Removal of Cyanotoxins for Water Security**

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Cyanobacterial toxins such as microcystin-LR (MC-LR), anatoxin-a (ATA), and saxitoxin (STX) represent a global environmental threat to ecosystems and drinking water supplies. Here we show the direct use of graphene as a rational interface for removal of MC-LR via interactions with the aromatic ring of the ADDA chain of the MC-LR toxin and the sp<sup>2</sup> carbon network of graphene. Mesoporosity of graphene is ideal for larger molecules and provided significant enhancements to both adsorption capacities and kinetics when benchmarked against microporous granular activated carbon (GAC) for the adsorption of all three cyanotoxins (MC-LR, ATA, and STX). Graphene showed superior adsorption capacity of 75.4 mg/g experimental, and 131 mg/g extrapolated for removal of MC-LR compared to 0.982 mg/g experimental and 0.973 mg/g extrapolated for GAC. Sorption kinetic studies showed graphene adsorbs 99% of MC-LR (1.6 mg/L) with pseudo-second-order kinetics in 30 min, compared to zero removal observed for GAC at 24 h at the same mass and concentration. Although noncovalent interactions typically rely on computational methods for mechanistic insight, nuclear magnetic resonance (NMR) titration was used to probe primary interactions between graphene and MC-LR adduct. With increasing graphene, the aromatic 7.2 ppm adduct peak broadened while aliphatic peaks remained sharp indicating interactions between the aromatic ring on MC-LR and graphene sp<sup>2</sup> orbitals are a dominant interaction. With rapid kinetics and adsorption capacities two to three orders of magnitude higher than GAC, it is anticipated that graphene will offer a novel molecular approach for removal of toxins and emerging contaminants with aromatic systems.

#### **1.08 Long-Term and Multigenerational Impacts from Early-Life Exposure to Contaminants**

### 1.08.T-01 Metabolic and Developmental Alterations in Fish Exposed to Waterborne Environmental Contaminants

**Hamid R. Habibi**, *Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada*

Studies in our group and investigators demonstrated the presence of many environmental contaminants in the water samples collected from three rivers in Southern Alberta, Canada. We detected synthetic estrogens, industrial chemicals, and pharmaceuticals close to the municipal area and natural hormones, pesticides, and herbicides in the agricultural areas. Significant increases in the female-to-male ratio and developmental abnormalities were observed in fish populations, linked to contaminants with hormone-like activity. To investigate the mechanisms underlying the adverse impacts of contaminants, we performed controlled laboratory experiments in which fish were exposed to environmentally relevant concentrations of some chemicals detected in the water, individually and in a simple mixture. We provided evidence that the adverse effects of contaminants are synergistic rather than additive when present in a mixture. Our results also provide evidence that exposure to environmental contaminants adversely affects the hormones of the brain–pituitary–gonadal axis, as well as inducing changes in lipid, amino acid, energy, carbohydrate, nucleotide, and cofactor/vitamin metabolism in the brain, liver, and testis of fish. The use of the microarray approach demonstrated significant changes in several canonical pathways, including cell cycle and proliferation, inflammatory, innate immune response, stress response, and drug metabolism. We observed developmental abnormalities following exposure to Bisphenol-A (BPA), phthalates, sulfolane, and zearalenone. Exposure to BPA during the embryonic stage at a critical period was found to disrupt brain development leading to hyperactivity at a later stage. On a positive note, we observed a potential beneficial action of probiotics against adverse actions of contaminants and the transgenerational effects of BPA. Elucidating the mechanisms and determining the source of contaminants can help with better screening, remediation, and implementation of best practices to reduce exposure to contaminated food and water.

### 1.08.T-02 Early Life Exposure to Endocrine Disruptors: Key Learnings from Metabolome Mapping of Rat Models

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Exposure to endocrine-disrupting chemicals (EDCs) in humans during pregnancy and early stages of life can impair normal brain development and reproductive function patterns, leading to severe pathologies later in life. Environmental studies have investigated bisphenol (BPA mostly) and phthalate developmental neurotoxicity. However, many EDCs such as pyrethroids, per- and polyfluoroalkyl substances (PFAS), organophosphate flame retardants, and plasticizers are hardly related to neurodevelopmental diseases, and new knowledge has to be gained. Metabolomics has shown to be a powerful tool in environmental toxicology in recent years: the key function of steroid and thyroid hormones, neurotransmitters, and lipids for brain development, homeostasis, and metabolism makes all of them potential candidates in the discovery of sensitive biomarkers for neuroendocrine toxicity in animals and humans. We, therefore, applied targeted and untargeted metabolomics to map the metabolic pathways mostly influenced by the exposure to six EDCs, namely bisphenol F (BPF), permethrin (PMT), butyl benzyl phthalate (BBzP), triphenyl phosphate (TPHP), perfluorooctane sulfonic acid (PFOS), and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) in rats during pregnancy and lactation. Hormone levels measurements are pivotal for *in vivo* mechanistic evaluation of EDCs' mode of action (MoA) and risk assessment. This study is specifically designed to detect and extend all the possible metabolic endpoints relevant for assessing neuro-endocrine toxicity EDCs mediated at the early stages of life.

### 1.08.T-03 Poly- and Perfluorinated Chemicals Alter Development and the Lipidome of Fathead Minnow Embryos

**Nancy D. Denslow<sup>1</sup>**, *Kevin J Kroll<sup>1</sup>*, *Anna Marquero<sup>2</sup>* and *John A Bowden<sup>1</sup>*, *(1)University of Florida, (2)Environmental Chemistry Dept., IDAEA-CSIC, Spain*

Per- and polyfluoroalkyl substances (PFAS) have been released into the environment through a multitude of industrial and consumer-based products. Per- and polyfluoroalkyl substances have become an important emerging concern worldwide, as these substances are found in biota, water, and sediments and constitute potential hazards to environmental and human health. They are known endocrine disruptors, causing infertility and hormone disruption in addition to immunotoxicity, hepatotoxicity, and cancer, but their modes of action are still not well-delineated. While it is generally thought that PFAS act through activation of the peroxisome proliferator-activated receptor (PPAR $\alpha$ ), the concentrations required for transactivation are higher (100  $\mu$ M) than levels normally found in blood. It is possible that alternative biochemical mechanisms are involved at concentrations that appear in the environment. To begin to understand the toxicity mechanisms *in vivo*, early life stage fish exposures (0, 0.5, 5, 50  $\mu$ g/L) were undertaken using dechorionated fathead minnow embryo. Exposures were conducted for 6 days (10 d postfertilization). Per- and polyfluoroalkyl substances altered proper heart development, heartbeat, and lipid profiles, suggesting activation of several different types of pathways in addition to PPARs. In RNAseq experiments with adults, the main pathways targeted included lipid related pathways, hormone biosynthesis, glucose metabolism, and respiratory chain, among others. These studies point to a main effect of PFAS on the less studied but fundamental biochemical pathways, including mitochondrial function, respiration, and metabolism. Per- and polyfluoroalkyl substances are persistent environmental hazards and how they perturb the endocrine system of aquatic organisms is an area of urgent research.

### 1.08.T-04 Transgenerational Effects of Ancestral Arsenic Exposure on Cognitive Performance and Dopamine Signaling Pathway in the Brain of Zebrafish

**Som Niyogi**, *Mahesh Rachamalla and Arash Salahinejad, University of Saskatchewan, Canada*

Arsenic is a priority environmental pollutant because of its toxicity to human and nonhuman species including fish. It is known to

cause neurotoxicity primarily by inducing oxidative stress and neural dysfunctions, and chronic exposure to arsenic has been suggested to impair various behavioural functions including cognition. The present study was designed to investigate the transgenerational effects of ancestral arsenic (As) exposure on the cognitive performance of zebrafish (*Danio rerio*). Adult zebrafish (6 months old; F<sub>0</sub> generation) were exposed to four different concentrations of dietary arsenic (0 [control], 30, 60, and 100 As µg/g dry weight; as arsenite) for 60 d. The cognitive behaviour of fish was then examined using a latent learning paradigm in a complex maze, which showed a dose-dependent adverse effect of As on cognitive performance. The As-induced cognitive impairment was associated with increased As accumulation, dopamine level, and oxidative stress in the brain relative to that in the control. Moreover, marked alterations of several genes involved in the signalling of dopamine, which regulates important fish behaviours including learning, memory, and reward-motivated behaviours, were also observed in the brain of As-exposed fish. Following behavioural assessments, As-exposed females from each treatment were mated with control males, and vice versa, to produce F<sub>1</sub> generations of fish that were maternally (ME) and paternally (PE) exposed to As, respectively. Subsequently, adult males and females from each F<sub>1</sub> generation ME and PE treatment groups were bred again to produce F<sub>2</sub> generations of the respective ancestral As treatment groups. Both F<sub>1</sub> and F<sub>2</sub> generations were raised to adulthood (6 months) in clean water and diet before assessing their cognitive performance as described above. A strong cognitive effect was observed in both F<sub>1</sub> and F<sub>2</sub> generations from the maternal lineage of ancestral As exposure, even at the low dose (30 µg/g). In contrast, in the F<sub>1</sub> and F<sub>2</sub> generations from the paternal lineage of ancestral As exposure, the cognitive impairment was recorded only at the highest dose group (100 µg/g). Increased oxidative stress and dopaminergic dysregulation were also observed in the brain of F<sub>1</sub> and F<sub>2</sub> generations of fish irrespective of the maternal or paternal lineage of ancestral As exposure. Our study indicates that ancestral As exposure causes transgenerational neurobehavioural effects in zebrafish, likely via epigenetic alterations.

#### 1.08.T-05 Effects of Environmentally Relevant Mixture Concentrations of Estetrol/Drospirenone in Zebrafish: A Multigenerational Study

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Natural and synthetic steroid hormones are widely found in aquatic ecosystems and have the potential for unintended consequences in the environment due to their ability to disrupt the normal endocrine function of wildlife. Notably, the synthetic estrogen ethinylestradiol (EE2), widely used in combined oral contraceptives, has been shown to be more potent than natural estrogens to aquatic organisms.

The natural estrogen estetrol (E4) was recently approved for use in a new combined oral contraceptive with the progestin drospirenone (DRSP). The aim of this study was to characterize and compare the endocrine disrupting potential of mixtures of E4/DRSP and EE2/DRSP to zebrafish, over multiple generations.

The Medaka Extended One Generation Reproduction Test (OECD Test Guideline No. 240) was adapted to zebrafish (*Danio rerio*). Fish were exposed over three generations (F<sub>0</sub>, F<sub>1</sub>, and F<sub>2</sub>) to different environmentally relevant mixtures of E4/DRSP or EE2/DRSP. Selected mixture concentrations represented increasing multiples (~1, 3, 10, and 30x) of the predicted environmental concentration of each substance.

EE2/DRSP mixtures: In the F<sub>0</sub> generation, fecundity was significantly reduced in fish exposed to 3.2/32 ng/L (*i.e.* ~ 10x environmental concentration). In the F<sub>1</sub> generation, at concentrations as low as 1/10 ng/L (*i.e.* ~ 3x environmental concentration), a significant change in gonadal development and a decrease in fecundity was observed. Vitellogenin synthesis was also significantly increased, and fish growth significantly decreased, at 3.2/32 ng/L (*i.e.*, ~ 10x environmental concentration).

E4/DRSP mixtures: Only the highest tested mixture concentration (~30x predicted environmental concentration) resulted in effects on the F<sub>1</sub> generation. Effects included an increase in the proportion of males. No impact on fecundity, growth, or vitellogenin synthesis was observed.

While a chronic exposure to environmentally relevant concentrations of EE2/DRSP severely impacted zebrafish population dynamics including an impairment of fecundity and gonadal development, relatively limited effects of E4/DRSP were observed in the zebrafish multigenerational exposure, with no impact on fecundity, growth, or vitellogenin synthesis. In conclusion, the data show that the combination E4/DRSP has a potentially more favorable toxicity profile to fish compared to EE2/DRSP and suggest that E4/DRSP may represent a more ecofriendly combined oral contraceptive.

#### 1.08.P Long-Term and Multigenerational Impacts from Early-Life Exposure to Contaminants

##### 1.08.P-Th058 Multigenerational Effects of the Novel Brominated Flame Retardant, 1,2,5,6-Tetrabromocyclooctane (TBCO), on Japanese medaka (*Oryzias latipes*)

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The novel brominated flame retardant (BFR), 1,2,5,6-tetrabromocyclooctane (TBCO), is a replacement option for major use BFRs that are being phased out of usage due to concerns about toxicity. Little is currently known about the toxic effects of TBCO; however, waterborne exposure has been demonstrated to induce toxicity in early life stage zebrafish (*Danio rerio*) and

dietary exposure has been demonstrated to impair reproduction in Japanese Medaka (*Oryzias latipes*). Maternal transfer is a mechanism by which developing offspring can be exposed to toxicants. Objectives of this research were to investigate effects of maternally deposited TBCO on development and reproductive success in three generations of Japanese medaka (F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>). Sexually mature fish (F<sub>0</sub> generation) were fed either a control diet or a low (40.6 µg/g) or high (1034.4 µg/g) diet containing TBCO for 21 d and three generations of embryos were reared to determine reproductive performance. Once the F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> generations reached sexual maturity, reproductive performance was assessed by use of a 21-d short term reproduction assay where fecundity and fertility were measured daily. Additionally, embryotoxicity was assessed during the final week of each reproduction assay. Molecular mechanism(s) of effect were investigated by use of enzyme-linked immunoassay (ELISA) and Quantitative Real-Time PCR. Concentrations of TBCO in eggs (F<sub>1</sub> generation) from fish given the low and high diets were 711.3 and 2535.5 ng/g wet weight, respectively, confirming maternal transfer of TBCO. Embryotoxicity was evident in the F<sub>1</sub> generation, but not the F<sub>2</sub> or F<sub>3</sub> generation. Cumulative fecundity of the F<sub>1</sub> generation in the low and high treatment were reduced by 33.9% and 33.3%, respectively, compared to the control. In the F<sub>2</sub> generation, cumulative fecundity of the low treatment returned to the level of the controls, but the high treatment was decreased by 29.8%. There was no decrease in cumulative fecundity in the F<sub>3</sub> generation compared to controls. This research ultimately provides insight into the long-term effects of TBCO as its usage is poised to increase. Maternally deposited TBCO causes embryotoxicity, and also causes multigenerational, but not transgenerational, decreases in fecundity of female Japanese medaka. Interestingly, recovery of fecundity was concentration dependent. Mechanistically, decreased fecundity was unlikely to have been caused by developmental reprogramming of steroidogenesis.

**1.08.P-Th059 The Effects of UV-P on the Model Fish Species, Zebrafish (*Danio rerio*) Following Early Life Stage Exposure**  
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Benzotriazole ultraviolet stabilizers (BUVSs) are a class of chemicals that provide protection from UV-A and UV-B light, specifically at 280-400 nm. These anthropogenic compounds are added to a variety of products including building materials, waxes, personal care products, and paints to prevent UV degradation, cracking, and yellowing as a result of sun exposure. Despite the fact that BUVSs have a widespread occurrence in the aquatic environment, little is known regarding their effects on aquatic organisms. In fish, studies have reported that the BUVS, 2-(2H-benzotriazol-2-yl)-4-methylphenol (UV-P), is an anti-androgen and an agonist of the aryl hydrocarbon receptor (AHR). The overarching objective of this research was to characterize the effects of UV-P exposure on the reproductive success of adult zebrafish following early life stage (ELS) exposure and assess any molecular mechanism(s) of effect(s). To conduct this, zebrafish embryos were exposed, by use of microinjection, to UV-P at 0 (DMSO control), 0.04, and 0.4 mg/g-egg. Approximately 24 embryos were reared until complete yolk sac absorption, where embryotoxicity was assessed daily, where mortality and malformations, including spinal curvature, yolk sac edema, and pericardial edema were recorded. Heart rate was measured at 48 h postfertilization. To assess effects of ELS exposure on reproductive performance, the remaining injected embryos were reared until sexual maturity. At sexual maturity, fish underwent a 21-d reproduction assay, where fecundity and fertility were assessed daily. Upon termination of the reproduction assay, fish were euthanized, and gonads, liver, pituitary gland, and blood were collected for analysis of molecular and biochemical endpoints. There were no significant effects of UV-P on mortality, heart rate, hatching rate, or developmental malformations. Early life-stage exposure to UV-P did not have a significant effect on fecundity, relative to the control. However, there was a significant effect on fertilization success. Fertility was decreased by 10.0% in the 0.04 mg/g dose and by 21.1% in the 0.4 mg/g dose, relative to control. Results of this study provide novel insight on the effects of UV-P on early life stage development of fishes, how early life stage exposure can affect reproductive success at adult stages. This study will aid in providing an assessment of the risks associated with BUVSs to fish species.

**1.08.P-Th060 Zygotic Exposure to Venlafaxine Leads to Long-Term and Multigenerational Impacts in Zebrafish**  
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Venlafaxine, a selective serotonin and norepinephrine reuptake inhibitor, is currently detected in surface waters receiving municipal wastewater effluent at concentrations in the parts per billion range. We have carried out several investigations to understand the impact of this antidepressant on developmental programming using zebrafish as a model. Our results revealed that zygotic exposure to venlafaxine impacts brain serotonin content, affecting neurogenesis in specific brain regions, including the hypothalamus. This also corresponded with altered behaviour, which we were able to rescue with exogenous serotonin treatment. As the hypothalamus is a key integrator of endocrine function, we focussed our studies on the impact of venlafaxine on the growth and stress axes in zebrafish. Zygotic exposure to venlafaxine reduced the specific growth rate in juveniles and compromised the stressor-induced cortisol response in adults. Sexually dimorphic responses were observed as adults in response to early-life exposure to venlafaxine, including diminished cortisol stress response and aerobic scope in females. We also observed multigenerational effects on neurogenesis and behaviour, as well as cortisol stress performance, in response to zygotic exposure to venlafaxine in zebrafish. Taken together, our results reveal that early-life exposure to venlafaxine affects neurodevelopment and disrupts the developmental programming of the endocrine axes in zebrafish. Although studies investigating the impact of environmentally relevant levels of antidepressants are sparse, our results reveal that venlafaxine deposition in the embryo either through maternal transfer or from environmental exposure may lead to long-lasting and multigenerational impacts in fish. While the mechanism remains to be elucidated, we hypothesize that these effects may be in part associated with the disruptions in the early development of the monoaminergic systems.

### 1.08.P-Th061 Dietary Exposure to the Flame Retardant, BDE-99, Induces Multigenerational Behavioural Alterations in Atlantic Killifish

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To truly evaluate the long-term impacts of exposure to environmental pollution, it is critical to assess multigenerational effects. Polybrominated diphenyl ether (PBDE) flame retardants are highly persistent and ubiquitous in the environment, yet little is known about their potential for multigenerational toxicity. Direct exposure to one of the two most dominant PBDE congeners, BDE-99, has been linked to various adverse impacts on development, neurobehaviour, and reproduction, but studies focusing on multigenerational effects are lacking. To address this, we tested whether exposure to BDE-99 has negative effects that propagate across generations using Atlantic killifish (*Fundulus heteroclitus*) as a vertebrate model system. Adult wild-caught killifish were fed control or contaminated diet for 64 d during breeding season with two concentrations of BDE-99, 37.5 and 150 ng/g fish wet weight/day. The exposed (F0) fish were spawned to produce the F1 generation, which was raised under uncontaminated conditions and subsequently spawned to produce the F2 generation. No significant differences in fecundity, fertilization rate, developmental morphology, hatch success, or larval survival were observed between treatments in any generation. To evaluate neurological impairment in the F1 and F2 generations, larval locomotor activity was tracked in response to alternating light and dark conditions. Parentally exposed F1 fish from some of the BDE-99 lineages expressed significant hypoactivity during the light phases, while results suggest that that behaviour is not altered in F2 larvae. At juvenile stages, novel tank diving tests were conducted to assess anxiety-like behaviour in the F1 and F2 generations. Results indicate an anxiolytic effect (greater percentage of time in the top zone of the tank) in the F1 fish parentally exposed to BDE-99; however, this behavioural effect was not propagated to the F2 generation. Archived juvenile brain tissues are being analyzed for genome-wide gene expression changes to infer potential mechanisms associated with observed behavioural phenotypes in the parentally exposed generation and generate hypotheses about other multigenerational neurotoxic effects of BDE-99.

### 1.08.P-Th062 Persistent Effects of Early Life Exposure of Zebrafish to Water from the Richelieu River (QC, Canada)

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Fish worldwide spawn in waters that are contaminated with complex mixtures of environmental chemicals. This may have important consequences on offspring, particularly if exposure occurs at a sensitive early life stage (ELS). Here we evaluated how ELS exposure to pesticide-contaminated river water affected the biology of a model organism, the zebrafish, as larvae and adults. Water samples were collected from an agricultural river (Richelieu River, QC, Canada) during the spawning season of listed fish in June 2019. Two surface water samples were collected at Chambly and Saint-Ours following heavy rain events when pesticide concentrations were the highest. Targeted pesticide analysis indicated that the Saint-Ours sampling site, located downstream of tributaries fed by agricultural drainage, had higher pesticide concentrations than the Chambly site. Zebrafish embryos (three replicates of 100 embryos each) were exposed to river water (Chambly or Saint-Ours) or laboratory water (control group) until 120 h postfertilization. Thereafter, zebrafish were raised to maturity in clean water. We found that exposure to river water delayed zebrafish hatching compared to controls but had no effect on larval survival or deformities. In adults that were exposed as embryos, the number of eggs produced was not different between the treatment groups. However, fertilization success for the fish exposed to river water collected at Chambly and Saint-Ours was lower than the control group (65, 72, and 81%, respectively). Overall, effects were more pronounced in the fish exposed to water from the more contaminated downstream site. These results increase our knowledge on the potential long-term effects of realistic mixtures of environmental contaminant exposure on ELS fish.

### 1.08.P-Th063 Embryo-Larval Toxicity and Molecular Toxicity Pathways of Maternally Transferred Hexabromocyclododecane (HBCD) in Fathead Minnows (*Pimephales promelas*)

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Hexabromocyclododecane (HBCD) is a brominated flame retardant that presents a threat to aquatic organisms as it can be maternally transferred and has been reported to hinder development and survival in early-life stage (ELS) fish. However, little is currently known about the molecular mechanisms that drive the toxicity of HBCD. This study examined the apical and molecular response patterns following exposure to maternally transferred HBCD in fathead minnow (*Pimephales promelas*) larvae at seven (whole transcriptome) and 14 (apical and physiological) days postfertilization (dpf). Transcriptomics analysis revealed dysregulation of pathways involved in membrane integrity (inhibition of calcium channel) and metabolic processes (downregulation of amino acid, glucose, and lipid biosynthesis), while the larvae reared for 14 d exhibited a significant decrease in survival in the highest (100 mg/kg) treatment condition. These results indicate that maternal transfer of HBCD is of concern in fish and exposed progeny may lead to the inhibition of membrane transport and disruption in metabolic processes, collectively resulting in energy depletion and subsequent mortality. Most importantly, what can be inferred from the current study is that



HBCD can be maternally transferred to embryos, where it elicits a potentially different mechanism of toxicity than previously suggested by waterborne studies.

#### 1.08.P-Th064 Transcriptomic Profile in Zebrafish (*Danio rerio*) Embryos Exposed to Environmental Concentrations of Glyphosate

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Glyphosate [N-(phosphonomethyl)glycine] is one of the most popular herbicides worldwide. The use of herbicides containing glyphosate as an active ingredient has intensified to combat herbicide-resistant weed species. Currently, glyphosate is present in 750 commercial herbicide products designed for intensive crop production, market gardening, and gardens in general. From the time that glyphosate was introduced into the market in 1970 until 2014, it has been estimated that 8.563 million kg of glyphosate has been produced worldwide. Globally, the use of glyphosate is increasing, and its residues have been found in drinking water and food products. The herbicidal mode of action of glyphosate results from the inhibition of a key enzyme (5-enolpyruvylshikimate-3-phosphate synthase) of the shikimic acid metabolic pathway that is involved in the synthesis of aromatic amino acids. This pathway is not present in mammals. However, aside from the toxicity attributed to some components of commercial glyphosate formulations, the controversy surrounding glyphosate toxicity remains unresolved, especially at environmentally realistic levels of exposure. Glyphosate residues have been identified in bodies of water adjacent to sites in which glyphosate herbicides have been applied. In natural aquatic environments, glyphosate concentrations have been found to range from 0.04 mg/L to 700 mg/L in different sites worldwide. Environmental standards in the United States limit the glyphosate concentration in water to 700 mg/L while the European Union has a more restrictive environmental standard of 28 mg/L. Therefore, the aim of this study was to evaluate the effects of glyphosate at environmental concentrations in zebrafish (*Danio rerio*) embryos. Embryos were exposed to 0, 1, 100, and 1000 µg/L glyphosate for 96 h, and mortality, heart rate, and hatching rate were evaluated. After the experiment, RNA was extracted from the embryos for transcriptional analysis. No mortality was recorded, and exposure to 100 µg/L and 1000 µg/L of glyphosate resulted in lower heart rates at 48 h. In addition, RNA-seq analysis revealed that glyphosate exposure induced subtle changes in gene transcription profiles. We found 30 differentially expressed genes; however, the highest glyphosate concentration (1000 µg/L) induced the greatest number of differentially expressed genes involved in oocyte maturation, metabolic processes, histone deacetylation, and nervous system development.

#### 1.08.P-Th065 Effect of Mifepristone on the Development of African Clawed Frog (*Xenopus laevis*)

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The aquatic environment is constantly polluted with substances that can affect the endocrine system of aquatic organisms. These compounds, known as endocrine disruptors (EDs), display diverse types of hormonal activities, including antiprogestogenic activity. However, there is limited knowledge about the effect of substances with antiprogestogenic activity. Therefore, the objective of the present study was to assess the effects of compounds with antiprogestogenic activity on the development of amphibians. Mifepristone, a synthetic steroid hormone commonly used in human medicine as an abortifacient, was chosen as a model compound with antiprogestogenic activity. African clawed frog (*Xenopus laevis*) tadpoles were exposed to mifepristone at three concentration levels (2, 22, and 215 ng·L<sup>-1</sup>). Moreover, a control group with DMSO (0.001 %) was included. The experiment started when tadpoles reached stage 47-48, according to Nieuwkoop and Faber (NF), and continued until stage NF 66 when metamorphosis was complete. Exposure to mifepristone did not affect the developmental rate, weight, length, and sex ratio of tadpoles. Mortality was within an acceptable range of 1–5% throughout the test, with no differences among the groups. Histopathological examination of the gonads and thyroid gland did not reveal any significant changes. Therefore, we can conclude that mifepristone did not have a negative effect on the development of the African clawed frog. However, in the highest tested concentration of mifepristone (215 ng·L<sup>-1</sup>), gene expression analysis revealed up-regulation of mRNA expression of nuclear progesterone receptor (*npr*), membrane progesterone receptor (*mpr*), and luteinising hormone (*lh*) in the brain-pituitary complex of exposed frogs at stage NF 66. Higher mRNA expression of *npr* was also found in frogs exposed to 22 ng·L<sup>-1</sup> mifepristone compared to the solvent control. These findings confirmed the antiprogestogenic activity of mifepristone in frogs because up-regulation of progesterone receptors occurs if progesterone availability in the body is reduced. This effect may have negative consequences for reproduction later in life.

#### 1.08.P-Th066 Transgenerational Adaptation to Ocean Acidification Determines the Susceptibility of Filter-Feeding Zooplankton to Nanosized Plastic

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The adaptability of marine organisms to the impending challenges presented by ocean acidification (OA) is essential for their survival, and the mechanisms underlying OA adaptation have been reported in several marine organisms. In the natural environment, however, marine organisms are often exposed to a combination of environmental stressors, but the interactions between different adaptive responses have yet to be elucidated. Here, we investigated the susceptibility of filter-feeding rotifers to short-term (ST) and long-term (LT) (≥180 generations) high CO<sub>2</sub> conditions coupled with nanoplastic (NPs) exposure (ST+ and LT+), and tested how their susceptibility to NPs can be affected by parental exposure to high CO<sub>2</sub>. The adaptive capacity of rotifers to elevated CO<sub>2</sub> caused differences in the ingestion and accumulation of NPs, resulting in a significantly different mode of

action on *in vivo* endpoints between the ST+ and LT+ groups. Moreover, rotifers exhibited unique transcriptomic profiles in the ST+ and LT+ groups, although they possess less genetic diversity. In the ST+ group, NPs induced apoptosis via an oxidative stress-induced lysosomal dysfunction pathway, whereas in the LT+ group, mTOR signaling pathways and calcium homeostasis conferred high adaptability to co-exposure of OA and NPs. Furthermore, the micro-RNA-mediated epigenetic regulation was strongly correlated with the varied adaptive responses between the ST+ and LT+ groups, revealing novel regulatory targets and pathways depending on their capacity to OA adaptation. Our results, therefore, indicate that history of exposure to increased CO<sub>2</sub> levels is an important factor that determines the susceptibility of rotifers to NPs.

## **1.09.A Micro (Nano)Plastics: Occurrence, Fate, Uptake, and Mechanistic Approaches to Understand Their Risk for the Environment and Human Health**

### **1.09.A.T-01 Eco-Corona Formation on Plastics: Impacts of Polymer Chemistry, Dissolved Organic Matter Composition, and Photochemical Weathering**

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Understanding of plastic particle fate in freshwater environments is still limited and experiments elucidating underlying factors remain scarce. Plastic particle chemistry, size, shape, density, as well as the freshwater composition all influence its fate. Aging of plastics, either by photochemical weathering or by adsorption of dissolved organic matter (DOM), which leads to formation of an eco-corona, further alters the physicochemical properties of plastics. However, the impact(s) of photochemical weathering on the physicochemical properties of plastic, and how this impacts subsequent adsorption of DOM and eco-corona formation, is often neglected. Here we conducted systematic studies on adsorption of a selection of ubiquitous DOM (Suwannee River Humic Acid, Suwannee River Fulvic Acid, and Suwannee River Natural Organic Matter) to a set of the most environmentally prevalent polymer types (polyethylene, polypropylene, polyethylene terephthalate, and polystyrene) in their pristine and photochemically weathered forms. Both pristine and photochemically weathered polymer films were characterized by water contact angle measurements (changes in polarity and charge), Fourier transform infrared spectroscopy (chemical changes), and atomic force microscopy (changes in physical surface roughness). Photochemical weathering induced decreases in polymer film thicknesses and water contact angles, and increases in surface roughness and carbonyl index. Using Quartz Crystal Microbalance with Dissipation (QCM-D) allowed for determination of adsorption and desorption of DOM to and from the polymer surface. We spun coated polymers onto the QCM-D sensor surface and exposed them to solutions of DOM in synthetic freshwater to monitor DOM adsorption over time. As a result of the physicochemical changes, photochemically weathered polymers displayed a decreased DOM adsorption rate and final mass in synthetic freshwater and suggests higher adsorbed masses on apolar versus polar surfaces. This information will help to understand the rate and extent of eco-corona formation for DOM on different pristine and photochemically weathered plastics. Ultimately, this will allow us to produce more realistically aged materials to be used in further experiments on fate of plastic particles.

### **1.09.A.T-02 Influence of Microplastics Composition and Algae Aggregates on Particle Settling Rates in Freshwater**

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To date, many studies have focused on the detection of microplastics (MPs) in natural waters, but only a few have been conducted to systematically understand the processes which drive MPs fate and distribution in freshwater ecosystems. This includes heteroaggregation with natural particles and small biota, and how these interactions affect transport in freshwater (e.g., settling dynamics). Freshwater snow (FWS), a mixture of algae and natural particles, is responsible for most of the flux of organic matter from the water surface to the sediments and can potentially act as a vector for MPs through the water column. Here, we systematically analyzed the settling rates of a variety of MPs in a plexiglass column, illuminated by a laser, and tracked with a stereoscopic camera system. Image sequences were acquired and analyzed with a tracing software to determine settling rates of particles in three test variants: 1) MPs of various size, density, and morphology; 2) FWS alone; and 3) MPs-FWS heteroaggregates. For each experimental set, our system allowed for the tracking of thousands of particles, which ensured statistical convergence of the results. Freshwater snow was created by mixing three strains of freshwater algae on a roller table over time. Our goal was to assess if the settling rates changed when MPs and FWS were aggregated together compared to their settling dynamics individually. For those MPs whose density is higher than water (PET, PLA), unsurprisingly, the settling rate increased with increasing particle size, but MPs fibers had a significantly slower settling velocity than the larger sizes of fragments. In all test variants, heteroaggregates settled faster than the individual MPs and significantly faster than the FWS alone, except for the case of MPs fibers. Conversely, those MPs whose density was lower than water (PP, PS) did not influence the settling rate of FWS no matter the MPs size. Collectively, we provide further insights on the settling rates of MPs and on a new experimental setup able to track thousands of particles per experimental run, as well as an indication as to how the presence of MPs can impact biogeochemical cycles through altering FWS settling. When MPs are incorporated into FWS, this faster settling rate will impact the flux of carbon, nitrogen, and phosphorus contained in FWS to the sediment, potentially having implications on productivity throughout the water column.

### **1.09.A.T-03 HDPE and PET Microplastics as Vectors of Microbial Pathogens**

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High density polyethylene (HDPE) and polyethylene terephthalate (PET) are two of the most widely used thermoplastic polymers in food packaging in Europe. In the last years, several studies have reported the presence of HDPE and PET microplastics (MPs) in a large variety of food products and beverages raising concerns about the health effects of HDPE and PET MPs after ingestion. In addition to that, recent studies have indicated that HDPE and PET MPs may serve as substrate for the growth of microbial pathogens. *Vibrio parahaemolyticus* (V) is a common foodborne pathogen commonly found in seafood. Consumption of seafood contaminated with *V. parahaemolyticus* is associated with acute gastroenteritis and serious septicemia. In the present study, we evaluated the potential toxicity of 5 µm HDPE (D50, commercially available) and 130 µm PET (D50, top-down cryomilled by centrifugal mill) MPs with and without *V. parahaemolyticus* biofilm (HDPE, PET, HDPE+V, and PET+V) to the human gastrointestinal (GI) system. Differentiated Caco-2 cells were exposed to different concentrations (1 to 100 µg/mL) of HDPE, PET, HDPE+V, and PET+V and effects on the GI barrier integrity (TEER, LY), cell viability (Alamar blue), and inflammatory responses (IL-6 and IL-8) were monitored from 1 h to 24 h of exposure. Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) were used to analyse *V. parahaemolyticus* colonisation on MPs. Scanning electron microscopy and CLSM analysis showed that *V. parahaemolyticus* formed a dense biofilm on MPs surface after 3 days of incubation. Results from the *in vitro* experiments indicated that despite not affecting Caco-2 cells viability at all tested concentrations, HDPE, PET, HDPE+V, and PET+V affected GI barrier integrity and induced inflammatory responses at high doses. Exposure for up to 24 h to HDPE and PET decreased GI barrier integrity, but higher effects were found in exposure to HDPE+V and PET+V. Similarly, HDPE and PET induced IL-6 and IL-8 secretion, but inflammation was higher in cells treated with HDPE+V and PET+V treatments. Results indicated that HDPE and PET MPs may act as vectors of *V. parahaemolyticus* and induce important cellular responses, triggering a cascade of events that can culminate in severe cell and tissue damage.

#### 1.09.A.T-04 Nylon 6,6 Microplastic Fibers Inhibit Differentiation of Human and Murine Airway Epithelium via Homeobox a5 Signalling

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Microplastics are a pressing global concern and are present in both indoor and outdoor air. Synthetic textiles shed fibers that accumulate indoors and this results in continuous exposure when indoors. Inhalation of high levels of microplastic fibers has been associated with interstitial and bronchial inflammation in nylon flock workers. However, exact health effects of microplastic fibers on the lungs are unknown.

Our aim was to assess the effects of nylon 6,6 (nylon) and polyethylene terephthalate (polyester) textile microplastic fibers on lung epithelial growth and differentiation.

We used alveolar and airway-type organoids derived from primary lung epithelial progenitor cells, isolated both from murine and human lung tissue, and incubated these with either nylon or polyester fibers or nylon leachate. In addition, mice received one dose of nylon fibers or nylon leachate intratracheally and 7 d later organoid-forming capacity of isolated epithelial cells was investigated.

We observed that in particular nylon microfibers inhibited developing airway organoids and not established ones, but nylon did not inhibit alveolar organoid formation. This effect was mediated by components leaching from nylon. Epithelial cells isolated from mice exposed to 75k or 150k nylon fibers or nylon leachate also formed fewer airway organoids, suggesting long-lasting effects of nylon components on epithelial cells. RNA sequencing analysis showed that the most prominently upregulated genes after exposure to nylon fibers included Homeobox a5 (*Hoxa5*). Inhibition of *Hoxa5* protein during exposure to nylon restored both human and murine airway organoid formation, confirming an essential role of *Hoxa5* in the effects of nylon.

Our results show that components leaching from nylon 6,6 may especially harm developing airways and/or airways undergoing repair. This suggests that airborne nylon may be most harmful to young children with developing airways and/or to people undergoing high levels of epithelial repair. These could be people with a chronic lung disease or even healthy individuals suffering from a seasonal respiratory virus infection. We therefore strongly encourage to look in more detail at both hazard of and exposure to microplastic fibers.

#### 1.09.P-Mo043 EnviroPlaNet Project: A Systematic Monitoring of Atmospheric Deposition of Microplastics in Spain

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Plastic pollution is a global concern. The world's production continues to increase yearly, reaching more than 367 million tons in 2020. Part of these plastics reach the ecosystems as microplastics (MPs). From all the environmental compartments, the presence of MPs in the atmosphere has been poorly studied. The objective of this work was the systematic monitoring of the deposition of MPs in areas of Spain with different characteristics (population, economic activities, and climate) using a common method for sampling, quantification, and identification during the four seasons of a one-year sampling campaign with the purpose of clarifying the role of the atmosphere in the fate and dispersion of MPs.

The collection of airborne MPs was performed using a set of passive collection made of steel and glass specifically designed to help QC/QA during handling and monitoring stages. The results showed that fibres were the dominant shape followed by fragments, filaments, microspheres, and films. Their sizes ranged from 14 µm to 4725 µm. The spectroscopic analyses performed using micro-FTIR (Fourier transform infrared) revealed that near 50% of microparticles potentially identified as MPs were plastic polymers while the rest were identified as cellulose (38%) and a 12% of microparticles lacking enough evidence for unambiguous identification. A total of nine different polymers were determined. The majority of fibres were identified as polyester (PES) while the fragments were PES, polyurethane (PUR), and polymethyl methacrylate (PMMA). Filaments and microspheres were dominated by polyethylene terephthalate (PET), while all films were polyethylene (PE). The atmospheric deposition of MPs ranged from 0 to 98.4 MPs m<sup>-2</sup> day<sup>-1</sup> with an average 27 MPs m<sup>-2</sup> day<sup>-1</sup>. Statistical analysis showed a positive correlation between the population of the study area and the average atmospheric MP deposition ( $R^2 = 0.87$ ). These results suggest a key role of the urban areas as sources of atmospheric MPs. Regarding environmental factors, a negative correlation was found between the average wind speed and the average atmospheric MP deposition ( $R^2 = -0.91$ ), suggesting that winds could clean the atmosphere from MPs. This is the first study of atmospheric deposition of MPs performed over a one-year period with four samples taken, one per season and covering all the Spanish peninsular territory and the Canary Islands.

**1.09.P-Mo044 Characterizing Human Exposure to Microplastics During Pregnancy** *Charles Rolsky<sup>1</sup>, Whitney Cowell<sup>2</sup>, Michelle L. Berger<sup>1</sup>, Greg Drozd<sup>3</sup>, Eleanor Medley<sup>4</sup>, Kurunthachalam Kannan<sup>2</sup>, Varun Kelkar<sup>5</sup> and Leonardo Trasande<sup>2</sup>*, (1)Shaw Institute, Blue Hill, Maine, (2)Department of Pediatrics, New York University, (3)Department of Chemistry, Colby College, Waterville, (4)Department of Population Health, New York University, (5)Plastic Oceans International, Malibu, California

Plastic production has been increasing, on a global scale, since the 1950s. This has led to an upsurge in pollution from larger plastics, termed 'macroplastics' (>5 mm), as well as micro- (1 µm–5 mm) and nanoplastics (<1 µm). As more plastic is produced, utilized, and discarded, more opportunities are created for plastics, particularly nano- and microplastics (NMPs), to reach human populations. Ambient air, honey, table salt, bottled water, and more have been identified as direct exposure sources to humans. A variety of threats have been associated with NMPs exposure that include both physical and chemical toxicity. Once theorized, research has now demonstrated NMP contamination of human tissues and organs, including lung, placenta, liver, and more recently, breast milk. The effects of this contamination remain to be thoroughly understood, but regardless, more research is needed to document this exposure, especially as it relates to at-risk individuals, such as infants. Three small studies have aimed to shed light on the presence of NMPs in human placentas. This organ is unique as it is accessible from healthy adults and provides insight into the health of the mother and infant alike. As delivery rooms utilize copious amounts of plastic items, detecting any source of plastic contamination becomes paramount. Thus, we aim to study suspected human exposure to NMPs during pregnancy by quantifying and characterizing NMPs extracted from 300 placental samples collected during the first 14 months of the study, specifically targeting the chorion side, which we hypothesize could contain NMP contamination. This sample size is 17 times more than any previous placenta research project so will more broadly represent contamination in the population. Our study integrates sociodemographic, behavioural, lifestyle, and dietary health assessments to link potential exposure to higher rates of plastic-wrapped or processed food consumption, for example. Two novel methods of contamination controls will be implemented to reduce and/or document sources of plastic contamination from the delivery room or within the laboratory conducting the extraction. One employs the creation of a contaminant database of all medical plastics involved in birth and placenta removal. The other involves an innovative housing for extracted samples to reduce opportunity for airborne contamination, a suspected reason why some studies reporting NMP counts may be inflated.

**1.09.P-Mo076 Visualization of Labelled Nanoplastics in Algae and Copepods, Using Stimulated Emission Depletion (STED)-Microscopy and Fluorescence Lifetime Imaging (FLIM)**

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The possible impact of nanoplastics (NPs, dimensions <1 µm) differs from microplastics (MPs), which are usually limited to the digestive organs of the affected organisms. Studies show that the rate of transport over cellular barriers increases as the plastic particle size decreases, and also the transportation process (through endocytosis or through paracellular routes) depends on the particle size.

The aim of this project was to develop a method to label and visualise nanoplastics in their interaction with marine primary producers and consumers. This entails both the possible adsorption and absorption, to study uptake mechanisms, retention rates, and possible accumulation.

The method development consists of different parts. First, the label-technique was optimised, using absorptive swelling as described by Karakolis et al. (2019), with the commercial dye 'IDye' (ex. 669 nm, em. 550 nm). This dye has shown extreme stability for fluorescence imaging purposes and is compatible with STED (Stimulated Emission Depletion) microscopy. Next, the suitability of this labelling technique for plastic-toxicity testing was checked. The acute toxicity of the dye toward algae and copepods was tested using OECD protocols. A dose-response curve was built around the assumed exposure concentration ( $1.4 \times 10^{-8} \text{ mg mL}^{-1}$ ), and showed that the EC<sub>50</sub> ( $0.0277 \text{ mg mL}^{-1}$ ), and the EC<sub>10</sub> ( $0.00836 \text{ mg mL}^{-1}$ ) for marine algae are located far above this. Furthermore, to account for false positives and negatives in image analysis, the leaching of the dye from the particles, and the longevity of the fluorescence of the particles, is analysed as a function of time. The effects of the labelling on the plastic particle characteristics were compared to the nonlabelled plastics, using FTIR, Single Particle Tracking (SPT), and a Tecan plate reader.

This study focused on nanoparticles smaller than 200 nm, the size range at which different particles can no longer be distinguished due to the resolution limit of optical microscopes. To enable the visualisation of these particles, we worked with the super-resolution STED microscope. We were able to acquire significant increases in resolution. To visualise the labelled nanoplastics in interaction with the autofluorescent, phytoplankton species, we used FLIM (Fluorescence Lifetime Imaging Microscopy). This technique allowed us to differentiate between phytoplankton species and plastics due to differences in the exponential decay rate of the photon emission.

### **1.09.B Micro (Nano)Plastics: Occurrence, Fate, Uptake, and Mechanistic Approaches to Understand Their Risk for the Environment and Human Health**

#### **1.09.B.T-01 The Biofilm Attached onto Microplastics is a Critical Factor Modulating Their Role as Vectors for Co-Occurring Pollutants**

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Microplastics (MPs) have increasingly raised concerns about their environmental impact in recent years. There is evidence that plastic particles are present in every ecosystem on earth, where they could negatively impact the biota. In addition, MPs have been suggested to act as vectors of pollutants by sorbing and desorbing contaminants from their surrounding environment. Ingesting contaminated MPs might impair key organisms in the food chain. Microorganisms may also colonize MPs, forming biofilms that may affect the sorption and desorption of pollutants from MPs due to their ability to metabolize chemicals or form a possible sorptive phase. A wide range of MPs, pollutants, and microorganisms can be found in wastewater treatment plants, suggesting that they can represent an important source of entry of polluted/biofouled MPs into the environment.

In this study, we explore the role of polyethylene MPs as vectors for triclosan (TCS) and how the biofouling of these MPs by biofilms growing onto them, the so-called plastisphere, affects this vector effect. Waters from influent and effluent from a wastewater treatment plant (WWTP), and an *Escherichia coli* culture were used to promote biofouling. We also evaluated the biological impact of biofouled and nonbiofouled MPs as vectors of TCS on the filter feeder *Daphnia magna*. The sorption and desorption of TCS were assessed using MPs biofouled for four weeks and then put in contact with TCS for four hours. Biotic experiments were also conducted to test *D. magna* survival in chronic exposure (21 d) to 0-400 mg MPs/L. At the highest concentrations tested, nonbiofouled MPs were toxic, but in all cases the toxicity of MPs was higher when biofouled in wastewater effluent. Regardless of the source of biofouling, both biofouled and nonbiofouled MPs exposed to TCS were capable of sorbing and releasing it, turning more toxic to *D. magna* than those not exposed to TCS. Triclosan-loaded MPs were also more toxic to *D. magna* when allowed to be colonized in wastewater effluent. The results indicated that MPs can transfer pollutants to the biota, causing toxic effects. Several factors could modulate this toxicity, including the nutritional status and the microbial community growing in the biofilm. In addition, it was observed that the exposure to TCS modified the composition of the colonizing community, selecting taxa with TCS-resistant and degrading abilities.

#### **1.09.B.T-02 Screening and Prioritization of Nano- and Microplastic Particle Toxicity Studies for Evaluating Human Health Risks – Development and Application of a Toxicity Study Assessment Tool**

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Concern regarding the human health implications that exposure to nano- and microplastic particles (NMPs) potentially represents is increasing. While there have been several years of research reporting on the ecotoxicological effects of NMPs, human health toxicology studies have only recently emerged. The available human health hazard data are thus limited, with potential concern regarding the relevance and reliability for understanding the potential human health implications. In this study we develop and apply a NMP toxicity screening assessment tool (NMP-TSAT) for evaluating human health effects studies against a suite of

quality assurance and quality control (QA/QC) criteria for both *in vivo* and *in vitro* studies. A total of 74 studies representing either inhalation or oral exposure pathways were identified and evaluated. Assessment categories include particle characterization, experimental design, and applicability for risk assessment, with critical and noncritical criteria organized to allow screening and prioritization. It is observed that the majority of studies evaluated using the NMP-TSAT have been performed on monodisperse particles, predominantly spheres ( $\approx 60\%$ ), consisting of polystyrene ( $\approx 46\%$ ). The majority of studies have tested particles  $< 5\ \mu\text{m}$ , with a minimal particle size of  $10\ \text{nm}$  and a maximum particle size of about  $200\ \mu\text{m}$ . The total assessment score (TAS) possible for *in vivo* studies is 52, whereas for *in vitro* studies it is 46, which is based on receiving a maximum score of 2 against 26 and 23 criteria, respectively. The evaluated TAS ranged from between 12 and 44 and 16–34, for *in vivo* and *in vitro* studies, respectively. Several key observations for strengthening future effects studies are identified, based on the results observed from the evaluation exercise, which includes a need for the generation and access to standard reference materials representative of human exposure to NMPs for use in toxicity test systems and/or the improved characterization and verification of test particle characteristics, and the adoption of study design guidance, such as recommended by OECD, when conducting either *in vivo* inhalation or oral ingestion toxicity tests.

#### **1.09.B.T-03 Risk Assessment of Microplastics in Soil Ecosystems Using Quality Criteria Screening and Data Alignment Methods**

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The occurrence and impacts of microplastics (MPs) on freshwater and marine ecosystems have widely been investigated over the past decade. However, less attention has been given to terrestrial ecosystems, even though they constitute major MP sinks due to the continuous use of plastic during common agricultural practices, such as mulching and greenhouse films, or sewage sludge application. The aim of this study was to characterize the risks of MPs in soil ecosystems, considering strict quality criteria and data alignment methods. For this purpose, we conducted a literature search to compile studies reporting measured environmental concentrations (MECs) of MPs in soil ecosystems, as well as toxicity data for terrestrial species exposed to MPs under controlled laboratory tests. The quality of exposure and effect data was screened after adapting QA/QC tools to soil data. Then, exposure and effect data were aligned to the standard MP size range ( $1\ \mu\text{m}$  to  $5000\ \mu\text{m}$ ), and effect data were rescaled to account for the polydispersity of environmental MPs and the bioaccessible MP fraction. Two species sensitivity distributions (SSDs) were constructed, one for volume and one for area as ecologically relevant dose metrics (ERMs), which trigger the effect mechanisms of food dilution and translocation, respectively. Using both SSDs, we calculated the Hazardous Concentrations for 5% of the species (HC5, with 95% confidence interval) for volume and area as ERM, and we compared them with the mean, minimum, and maximum MECs of MPs reported for soils with different land uses. The HC5 and 95% confidence limits obtained for volume and area as ERM were  $2.3 \times 10^7$  ( $8.9 \times 10^5 - 2.4 \times 10^9$ ) and  $6.2 \times 10^8$  ( $3.8 \times 10^7 - 3.5 \times 10^{10}$ ) particles/kg of soil (dry weight), respectively. The most sensitive species in both SSDs was the earthworm *Aporrectodea rosea*. When plotting the MECs together with the HC5 and the confidence limits for volume and area, we observe that 10 and 22% of the mean and maximum MECs exceed the HC5 value obtained for volume, while no MECs exceed the HC5 value obtained for area. Our results indicate that soil invertebrates might be at risk at 10–22% of the locations, globally. The MECs exceeding the HC5 value for volume include agricultural soils with various potential MP sources, but also forests and coastal soils. Based on the outcomes of this work, we conclude that soil ecosystems seem to be at higher risk due to the presence of MPs compared to surface waters and freshwater sediments.

#### **1.09.B.T-04 Short- and Long-Term Toxicity of Polyhydroxybutyrate Nanoparticles to the Freshwater Cnidarian *Hydra viridissima***

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The booming of manufacturing affordable plastic goods since the 1950s gave rise to an era of disposable items, powered by consumerism and convenience, which resulted in one of the greatest environmental concerns of our times. The improper use and disposal of petroleum-based plastics resulted in its accumulation in the environment with increasing reports of biological effects to biota. This motivated research on development of more sustainable alternatives, such as bio-based biodegradable polymers.

Recently, due to its biodegradability and physicochemical properties, polyhydroxybutyrate (PHB) has received growing interest. These innovative materials possess more attractive properties than conventional alternatives. Although PHB is currently available commercially, little is known about its environmental impact and effects on freshwater organisms.

In this context, this study aimed to fill the existing knowledge gap about the ecotoxicity of nanosized PHB plastic particles (PHB-NPLs) ( $\sim 200\ \text{nm}$ ) focusing on lethality, morphological changes, and feeding behaviour of the freshwater cnidarian *Hydra viridissima*, a promising model species for aquatic toxicology studies owing to the wide array of endpoints easily measurable and reported sensitivity.

#### **1.09.P-Mo045 Multigenerational Effects of Microplastics to Springtail**

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Environmental persistence of microplastics is ubiquitous and problematic. Despite that research on soil ecotoxicity of microplastics has been increased, the relationship between microplastics exposure to soil environment and springtail health has been insufficiently investigated. The purpose of the present study was the assessment of multigenerational effects of microplastics to springtail. The natural soil was amended with  $16\ \mu\text{m}$  of HDPE microplastics and *Folsomia candida* was exposed to the

contaminated soil by following OECD test guidelines No. 232. Through exposure to F2 generation, mortality, reproduction, and growth, damages in digestive system were examined. It was observed that HDPE exposure mainly caused decrease of survival and reproduction rate and inhibitions in microvilli and mitochondrial membrane in the intestinal tissues into continuous exposure group. These results indicated that soil collembola health can be affected by microplastics pollution.

#### **1.09.P-Mo046 Artificial Plastic Aging as a Framework for Microplastics Ecotoxicity Evaluation**

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Microplastics are widely distributed in aquatic and terrestrial environments, but up to now less is known on ecotoxicological impacts under realistic conditions. Research so far has focused mainly on the impacts on organisms by virgin plastic fragments or beads. However, plastics found in the environment are mainly a mixture of different polymers and additives and are degraded during aging processes, particularly. Although especially oxidized degradation products might escape from plastic materials during aging, there is a lack of information on potentially adverse effects on aquatic biota. The latter is of particular interest as oxidized chemicals might become more water soluble due to higher polarity and are therefore more bioavailable.

The present study focused on plastic leachates of polystyrene (PS) and polylactic acid (PLA), which were derived from alternating stress by hydrolysis and UV radiation. Test specimens of PS, PLA, or PLA/PS layers (each 50%) were alternately maintained in water at 45 °C for five days and UV radiated at 45 °C for two days, for a total of eight weeks. Ecotoxicological effects of potentially generated degradation products of plastics in the storage water (natural spring water) were detected by algae growth inhibition tests with *Desmodesmus subspicatus*.

Results clearly indicate inhibitory effects on algae growth by contaminants in the storage water of stressed plastics with increasing growth inhibition of proceeding hydrolysis and UV stress times. Here, different polymers cause variable responses of algae growth. First analyses indicate dissolved monomers and the degradation products of polymers as possible driver of detected ecotoxicological effects, since detected microplastic particles do not seem to harm algae. The existing data highlight the relevance of plastic aging as a framework for microplastic ecotoxicity evaluation and allow a proof of concept.

#### **1.09.P-Mo054 Leveraging Physiology and Behaviour to Better Understand Exposure, Uptake, and Elimination of Micro- and Nanoplastics (MNP) in Pelagic and Benthic Species within the Context of Quantitative Risk Assessment**

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Significant ecotoxicological data have been developed for micro- and nanoplastics (MNPs). However, several challenges to interpreting these data within the context of ecological risk assessment (ERA) remain. One such challenge is the selection of species for use in derivation of ecological protection criteria (EPC). Previous studies have proposed EPC values; however significant variability in species sensitivity across the range of MNPs exists, resulting in significant uncertainty in the application of derived EPCs as well as in identifying gaps in species sensitivity distributions (SSDs).

The objective of this work is to systematically compile and evaluate physiology and behavioural data for freshwater and marine species for use in ecotoxicological study design, identification of sentinel species for ecosystem health and quality monitoring, and ERA. Data are compiled from existing databases (e.g., Traitbank, Biological Traits Information Catalogue (BIOTIC), World Register of Marine Species (WoRMS), COPEPEDIA/COPEPOD, FishBase, and FishTraits). Life-history traits are used to describe habitat and motility. A trophic coding system is used to categorize species based on feeding mode and ecosystem functional roles. A trophic guild is then assigned based on species food source and feeding mode. Particular attention is directed toward benthic invertebrates given the likely deposition of MNPs in sediments and the relative paucity of benthic ecotoxicity studies. Using existing systems, benthic invertebrates are coded according to food source, diet, food type/size, and feeding mode. Existing MNP studies (biomonitoring and ecotoxicity) will be reviewed to identify trends in studied trophic guilds and compare them with trophic guild classifications likely to be most relevant for MNP exposure.

A biologically relevant framework is proposed against which the relevance of existing (and developing) MNP test materials and future studies can be evaluated. This can provide a systematic basis for the inclusion or exclusion of species or materials for the purpose of ERA in various environmental compartments. Data can also be integrated into risk assessment frameworks, providing guidance on species selection for ecotoxicity studies. Finally, species which have desirable traits for biomonitoring may also be identified based on their biological and physiological traits as well as abundance, distribution, and where possible the availability of established ecotoxicity testing methods.

#### **1.09.P Micro (Nano)Plastics: Occurrence, Fate, Uptake, and Mechanistic Approaches to Understand Their Risk for the Environment and Human Health**

##### **1.09.P-Mo047 Soil Ecological Risk Assessment of Microplastics Based on Species Sensitivity Distributions**

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Microplastics threaten the soil ecosystem causing adverse effects on organisms such as mortality, growth inhibition, and physiological factors, etc. This study aimed to conduct soil ecological risk assessment for microplastics based on the probabilistic approach. Based on data from previous studies, the species sensitivity distributions (SSDs) and environmental exposure distributions (EEDs) were estimated. In SSDs, hazardous concentration for 5th percentile of species (HC<sub>5</sub>) and HC<sub>50</sub> in soil

ecosystem was derived as 88.181 and 2656.527 mg/kg soil, respectively. In EEDs, a 95th percentile upper concentration of microplastics in agricultural land was estimated as 667.752 mg/kg soil. As a result of comparison of EEDs and SSDs, the overlap of them has been identified. These results suggest that microplastics threaten the soil ecosystem; accordingly, the establishment of a countermeasure for protecting the soil ecosystem from microplastic pollution is needed. This study can provide the basic information for the establishment of a soil management strategy for microplastic pollution.

#### **1.09.P-Mo048 Physiologically Based Kinetic (PBK) Modelling for Human Exposure to Micro- and Nanoplastics**

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Micro- and nanoplastics (MNPs, plastic particles <5 mm) are increasingly found in our environment and our food. Humans can be exposed to MNPs via several routes including inhalation, food consumption, and dermal contact, potentially imposing adverse effects on human health. Physiologically based kinetic (PBK) models estimate internal concentrations over time in human organs based on absorption, distribution, metabolism, and elimination (ADME) processes. PBK models have become essential tools since they allow a mechanistic framework to understand the internalisation of MNPs. However, PBK models for human exposure to MNPs are lacking. Typically, relevant processes and empirical data (*in vitro* and *in vivo*) are required to parameterise PBK models. Therefore, the aim of the present study is to summarise (the existing data on) ADME processes and discuss possibilities to develop PBK models for MNPs.

An extensive literature search was conducted on ADME information on PBK modelling for MNPs. A total of three relevant modelling articles on MNPs were found. Multiple relevant reviews and experimental articles regarding MNPs were also included. Absorption from the gastrointestinal tract (GIT) is most likely to occur via transcytosis in M-cells. Translocation in GIT depends on particle size, while the scientific consensus on cut-off values appears to be lacking. Particles above 1 µm in the lung are likely to be retained by the mucus layer and enter the GIT via mucociliary clearance. Microplastics smaller than 1 µm can be translocated into the systemic circulation or lymphatic system. Currently, modelling absorption via GIT and lung as first-order kinetics seems most appropriate. Empirically derived tissue-to-blood distribution coefficients can describe the distribution of MNPs between tissue and blood. The reticuloendothelial system plays a major role in the uptake of MNPs and translocation into the lymphatic system. Elimination of MNPs is regarded as negligible. It is worthwhile to develop experimental assays to determine MNPs concentrations in human blood and tissues. Since PBK modelling heavily relies on empirical data, generating consistent datasets (absorption, distribution and validation for the same size range) helps develop and validate PBK models for MNPs.

#### **1.09.P-Mo049 Shape Plays an Important Role in the Ecotoxicity and Environmental Aging of Microplastics**

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Microplastics represent a very heterogeneous group of pollutants, distinguished not only by their variable sizes and materials, but also by their shapes. Recently, researchers revealed that shape plays an important role in the ingestion of microplastics by animals, but how their shape affects other processes is unknown. In this context, the aim of this study was to investigate the interactions with aquatic macrophytes, adhesiveness, and environmental aging of polyethylene microplastics of the same size but different shapes, namely fragments, spheres, and films.

The investigated microplastics were of similar size (about 100 µm), and none of them had any effect on the growth rate of the aquatic macrophyte *Lemna minor* and on the chlorophyll *a* content. On the other hand, the fragments significantly reduced the length of the roots. Comparing the bioadhesion of fragments, spheres, and films, it was found that significantly less spheres adhered to the plant biomass ( $0.28 \pm 0.11$ ,  $0.02 \pm 0.02$ , and  $0.15 \pm 0.06$  microplastics/mg, respectively). The lowest amount of biofilm was developed on films, while the analysis of chlorophyll *a* content within biofilm showed that there were significantly more phototrophic microorganisms within the biofilm developed on fragments than on spheres and films. Our results shed light on the importance of the shape of microplastics for their interaction with the surrounding environment; spherical microplastics showed the greatest difference in the tested parameters compared to fragments and films. They also do not represent environmentally relevant microplastics, which is why the use of spherical microplastics in environmental microplastic research should be reconsidered.

#### **1.09.P-Mo050 Investigating the Influence of Microplastic Morphology and Surface Characteristics on Nitrogen Transformation in Floating Treatment Wetlands**

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Floating treatment wetlands are used for nitrogen removal in urban and agricultural runoff, sources which also contain high concentrations of microplastics. In wastewater treatment plants, the addition of microplastics has been found to influence the microbial communities performing similar nitrogen transformation and removal processes, raising concerns over the influence of microplastic pollution on the microbial communities in floating treatment wetlands.

To assess the impact of microplastics on nitrogen transformation, microplastics were incorporated into microcosms with root samples collected from mature floating treatment wetland mats. Nitrification microcosms were evaluated in aerobic conditions over 6 h and denitrification microcosms were flushed with nitrogen gas to promote anoxic conditions and monitored over 48 h in order to calculate potential nitrification and denitrification rates. Experiments investigating the impact of polyethylene and polystyrene microplastics at 30 µm and 200 µm size ranges showed a decrease in the rate of denitrification at the larger size range for both polymer types and an increase in the rate of denitrification for polystyrene at 30 µm. For the nitrification experiments,



polyethylene at 30 µm showed a decrease in the potential nitrification rate. While these trends show microplastics can influence nitrogen transformation in wetland systems, the mechanisms influencing these observed changes in microbial activity have yet to be defined. Further, these experiments were conducted using unweathered microbeads which are not representative of environmental microplastic samples.

To work toward more environmentally relevant samples, microplastics were created using milling techniques followed by UV weathering. After each milling and weathering step, microplastics were evaluated to assess size ranges, surface charge, and surface roughness. The study of microplastic surface characteristics, in addition to chemical properties, may elucidate the interactions between microplastics and microorganisms. This work will provide a better understanding of the impact of morphology and surface characteristics in addition to particle size gradients on changes in nitrogen transformation rates in wetlands as well as begin to explore some of the primary mechanisms by which microplastics are influencing wetland microbial communities.

#### **1.09.P-Mo051 Fate of Microplastic Particles in Stream Mesocosms**

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The amount of plastic litter in aquatic environments is increasing worldwide. Plastic residues enter the hydrological cycle in a wide range of sizes and break down gradually to tiny particles (<5 mm) called microplastic (MP). A distinction is made between primary MP (pMP), which are intentionally produced for industrial applications and personal care products, and secondary MP (sMP). Secondary MP are breakdown products of larger meso- (5–25 mm) and macro- (>25 mm) plastics. Most sources of MP pollution are land based, with car tire debris and artificial turfs being a major factor. These particles find their way into the aquatic ecosystem either during surface run-off events or via wastewater treatment plants. As a result, streams and rivers receive significant amounts of plastic debris. There is, however, still a major lack of knowledge about MP's fate and especially its retention by aquatic plants in freshwater systems. Therefore, the impact of macrophytes on the fate of MP particles was investigated using 12 artificial streams at the Eüßerthal Ecosystem Research Station (EERES, Eüßerthal, Germany) comprising 3.5 m long stream channels, fed by stream water from a nearby stream. Polystyrene (PS) and polyvinyl terephthalate (PET), two of the most common MPs, were chosen as model sMPs. We used three vegetation densities (0%, 25%, 100%) of the macrophyte *Elodea nuttallii*. We hypothesized that the retention of MPs is positively correlated with *Elodea* density. Analyses to (i) evaluate the longitudinal transport of MP within streams by means of water and sediment samples, and (ii) assess the retention of MP by macrophyte patches of different densities are currently ongoing. With this study we aim to get an insight on how two of the most commonly found sMP are transported within slow flowing streams and to what extent these particles are retained by the macrophyte *Elodea nuttallii*, since the retention of particles can also create a potential risk for organisms inhabiting these vegetated zones.

#### **1.09.P-Mo052 Modelling Environmental Microplastic Fate and Exposure**

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Exposure assessment is the basis of risk assessment for microplastic particles (MP). Since small microplastic and nanoplastic particles are almost impossible to detect, and their concentrations can only be estimated by interpolating in time and space, retrospective risk assessments must rely on modelling. This requires a holistic modelling approach, linking sources, transport, fate, and exposure of MP. In this research, we will develop and validate a range of microplastic transport and fate models, encompassing different spatial and temporal scales. The first step will be to develop a harmonized microplastic emission model, followed by the development of a 'unit world' multimedia model. Microplastic fluxes generated by this multimedia model will serve as input for a detailed spatiotemporal river transport model. While previous models were usually parameterized to simulate only the behaviour of monodisperse spherical microplastic particles, we will apply new approaches using probability density functions (PDFs). Using these PDFs we will be able to capture the full multidimensionality and diversity of environmentally relevant MPs as they exist in nature. Modelled MP-PDFs can then be tuned to threshold effect concentrations to characterize ecological risks in environmental systems. The toolset presented enables us to prospectively assess risks for decades to come.

#### **1.09.P-Mo053 Quantifying Environmental Emission and Risk of Microplastics in a Semi-Enclosed bay: A Tokyo Bay Case Study**

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In recent years, there has been growing concern about the impact of microplastics (hereafter MPs) on marine ecosystems, and political and social concerns have increased both domestically and internationally. The number of MP related studies in the marine environment is increasing every year, including monitoring in the environment, environmental behaviour, development of analytical methods, and ecological effects. However, there are few studies on quantitative assessment of MPs that contribute to develop realistic strategies for risk-based management for MPs. In this study, we reviewed domestic and international literature related to environmental risk assessment of MPs and quantify MP emission and environmental risk in the Tokyo Bay. A literature review was conducted to identify the latest trends in research related to the environmental emission estimation of MPs and the actual situation of MP concentration in Japan. To conduct a trial environmental risk assessment for Tokyo Bay, MP emission into

Tokyo Bay was calculated for MP contained in products, synthetic fibers derived from laundry, and tire wear particles. As one attempt to estimate a threshold concentration for the use in risk characterizations, we devised an estimation method for SSD using a hierarchical Bayesian model, which can quantitatively take into account the characteristics of MPs and attempted to estimate the hazardous concentration (HC5). In this talk, as a case study of Tokyo Bay, the results of environmental emission estimates of the selected sources and model analyses, as well as the risk estimates based on monitoring data of MPs, will be presented.

#### **1.09.P-Mo055 Comparison of Species Sensitivity Distribution Methods for Risk Assessment of Microplastics**

**Sara J. Hutton**, Kenia Whitehead, Janet Anderson and Philip E Goodrum, GSI Environmental, Inc.

Most papers published on ecological risks of microplastics (MP) are based on laboratory studies with limited exposure profiles or field studies that focus on the occurrence of MPs in aquatic organisms. However, the current scientific literature and knowledge regarding MP toxicity is sparse and often challenging to interpret. Examples of challenges with MP studies include incomplete/inconclusive reporting on microplastic characterization, and uncertainty in dose ranges (e.g., particle counts) due to particle aggregation. Given the limited number of high-quality studies that can be used in risk assessment, most efforts to quantify MP risk have lumped MPs into a single class rather than differentiating them based on a specific property (e.g., particle shape, polymer type). While this approach may currently be the most feasible, it may also be too simplistic and potentially under protective. In the past decade, there has been an increase in research and regulatory activity directed at supporting MP risk assessments. In 2016 the State of California, USA passed legislation requiring a comprehensive literature review to determine if a risk assessment could be conducted for human health (via drinking water) and aquatic organisms. California concluded that while there were insufficient data to conduct a formal risk assessment for human health, sufficient data were available to generate species sensitivity distributions (SSDs) based on no observed effect concentrations (NOECs) or lowest observed effect concentrations (LOECs). The SSDs were used to define four threshold levels; however, given the small sample sizes and well documented limitations associated with NOECs/LOECs, the confidence intervals calculated for each threshold almost entirely overlap. An alternative approach, first developed by USEPA's Office of Pesticide Programs to support ecological risk assessments of pesticides, and subsequently applied to site assessments of legacy contaminants, utilizes the full dose-response curve (DRC) rather than selected points of departure. The DRC method produces a composite DRC and confidence interval that reflects the range of different shapes of DRCs. Here we compare the threshold values for MPs when SSDs are constructed using the NOEC/LOEC approach and when the same study data are integrated using the DRC approach. We discuss the advantages and limitations of the approaches and demonstrate the implications of grouping MPs into subclasses based on common properties.

#### **1.09.P-Mo056 Bioenergetic Status of Human Intestinal Caco-2 Cells After Exposure to Simulated Environmental Nanoplastic**

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The ubiquitous human exposure to nanoplastic (NP) in our daily life increasingly raises concerns regarding our health. Currently, it is difficult to evaluate effects of NP in real-life exposure as substantial studies exposed human cells to pure nanopolymer particles with rather high concentrations, which cannot represent complicated NP samples suffering weathering in our living environment. In this study, the bioenergetic effects of four simulated environmental NP samples on human intestinal Caco-2 cells were investigated. To this aim, big micro-PET (polyethylene terephthalate) particles were mechanically milled into a lower size range sample (M-PET) with multiple shapes. Then the M-PET particles and a polystyrene (PS) mixture (100 nm and 700 nm, mixed) were irradiated by ultraviolet (UV) light for 1273 h, corresponding to 15 months of central European solar irradiance exposure. After weathering procedures, both virgin and UV-weathered M-PET samples were filtered by 0.8-µm filter to obtain nano-PET particles with size less than 800 nm. Subsequently, Caco-2 cells were exposed to nano-PET and nano-PS samples with and without UV weathering at realistic exposure levels ( $10^1$ - $10^6$  particles/mL) for 48 h. The mitochondrial respiration and glycolytic parameters of exposed cells were measured by Seahorse XF96 Analyzer. Based on these results, the harmful impacts of nano-PET on cellular bioenergy were stronger than those of nano-PS. Basal respiration, spare respiratory capacity, proton leak, and basal glycolysis were stimulated by stress from exposure to both virgin and UV-weathered nano-PET samples. Comparing virgin and UV-weathered nano-PET, the negative effects on mitochondrial respiration were alleviated while anaerobic glycolysis was enhanced for UV-weathered PET. Similarly, mitochondrial functions were more sensitive to virgin nano-PS while basal glycolysis was more vulnerable to UV-weathered PS sample. This research is the first to study bioenergetic responses of simulated environment NP samples on human health. It highlights that effects between virgin and weathered NP are different at a bioenergetic level, which has important implications for the risk assessment of NP on human health.

#### **1.09.P-Mo057 Macroplastics to Microplastics: The Production of Microplastic Particles from Large Debris in Freshwater Systems**

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Plastic pollution poses one of the greatest environmental challenges to date, mainly due to the microplastics produced. Microplastics can be produced from macroplastics in aquatic systems due to mechanical abrasion or weathering, UV degradation or photolysis, chemical degradation, and hydrolysis. As a result, it is extremely challenging to predict or model the abundance of

microplastic particles entering the environment. A common misconception of plastic debris is that it takes hundreds to thousands of years to degrade or mineralize. This underestimates the fragmentation that can occur along this period, contributing to varying rates of microplastics being produced over much shorter time scales. Previous work has identified that within a salt-marsh habitat, macroplastics can start producing microplastics in as little as eight weeks, with biofilm development occurring around week 4. In our assessment, we buried macroplastics ( $n = 5$ /polymer type) that represent common polymers found in the environment (polyethylene, polystyrene, polypropylene, polyethylene terephthalate, and polyvinyl chloride) and natural cellulose debris ( $n = 3$ /polymer type) in stream sediments from urban and forested areas to track the rate of fragmentation and biofilm development over the course of a year. To date, we have found through mRaman analysis that macroplastics from four of the five polymers started producing microplastics in as little as two weeks, faster than that observed in salt marshes in coastal systems and culturable bacterial communities in the same period, indicative of biofilm development. With a large emphasis on microplastic research focused on marine systems, it is important to note that ~80% of plastic debris comes from inland waters (streams, lakes, and rivers). With the fragmentation rate of microplastics being faster in these systems, it warrants more attention to the implications of microplastic pollution in freshwater habitats and what bacteria are preferentially selecting these polymers as a substrate.

#### **1.09.P-Mo058 Mapping and Quantifying the Various Sources of Microplastic Pollution in the Elbe River Basin**

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Microplastics (MPs) are found in many different environments. The aim of the present study was to quantify and map sources of MPs pollution in the Elbe River basin, which is the fourth largest river basin in Central and Western Europe. We identified the most relevant sources of MPs, including tyre wear, synthetic textile fibres, paint chips and plastic beads from cosmetics. The mapping was based on location-specific proxies, e.g., the per capita MP emissions from different sources combined with the population density of a given agglomeration. Methods were developed to include factors affecting the MP release process to produce more realistic estimates. For example, fabric type and washing temperature are both influencing the amount of microfibrils released, and the amount of tyre wear particles released varies considerably from one vehicle to another when driving on different types of roads. The results of this study will be used as input for modelling the fate of MPs in the Elbe River basin. For this, we will use the “Exposure to Pharmaceuticals in the Environment” model (ePiE) which originally was developed for calculating concentrations of active pharmaceutical ingredients (APIs) in surface waters. The model will be adapted to simulate the advective transport and degradation of MPs in rivers to provide a more comprehensive understanding of the sources, fluxes, accumulation areas, and fate of the MPs in rivers.

#### **1.09.P-Mo059 Wear and Tear of Synthetic COVID-19 Face Masks in an Artificial Wave Mesocosm System**

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The coronavirus pandemic prompted the extensive use of disposable face masks made of plastic polymers, and their use in public areas was mandatory in many countries. Unfortunately, their widespread use also led to their introduction into the environment through inappropriate disposal, i.e., littering or unintended loss. Being durable, they can remain in the environment for decades.

Though plastic pollution is one of the most pressing environmental issues of our time, little is known about degradation processes under natural conditions and the coronavirus pandemic has added a new item of concern. Mechanical abrasion plays an underestimated role in the formation of secondary microplastics of all plastic polymers and in this context, the degradation process of three different mask types (KN95, FFP2, and surgical face masks) was investigated in the semiterrestrial zone of the artificial wave mesocosm system at the German Environment Agency’s field station, where seminatural conditions can be simulated on a larger than laboratory scale.

For each face mask type, 3x5 masks were exposed to a stone-gravel-shore in two wave mesocosms and one unmoved body of water as control, for approximately 200 days. Loss of mass, but also changes in surface structure of the three different mask types, were monitored.

Loss of mass varied between the different masks with approximately 10%–15% for the KN95 masks, 4%–4.5% for the surgical face masks, and 4%–6% for the FFP2 masks; thus not all masks display the same durability. All masks displayed clear signs of wear and tear, but especially the FFP2 and KN95 masks showed distinct signs of peeling. When the surface of the masks was viewed under a microscope and white light interferometer, fibers appeared fairly durable with only slight defibration or fraying of the outer melt-blown, nonwoven fabric layer, that was primarily exposed to the gravel shore.

#### **1.09.P-Mo060 Integrated Toxicological Analyses of Marine Micro- and Nanoplastic Particles Using *in vivo* and *in vitro* Bioassays**

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Microplastics are detected at the base of the food chain, posing a potential threat to marine ecosystems but also raising concerns about potential impacts on humans due to adsorbed chemicals and additives exhibiting toxicity. Target-, suspect, and nontarget screening are used to identify the chemical composition of microplastics. However, due to the vast number of microplastics-

related toxicants and the cocktail effect caused by the mixture of chemicals, chemical analyses are insufficient for hazard assessment. Effect-Directed-Analysis (EDA) represents an integrative assessment of toxic fractions or chemicals, that combines biotesting, fractionation procedures, and chemical analysis methods. Effect-Directed-Analysis provides an accurate characterisation of the toxicity of bioactive chemicals using *in vivo* and *in vitro* bioassays.

This study is associated with the European research project “Response: Towards a Risk-Based Assessment of Microplastic Pollution in Marine Ecosystems” with the objective to develop a monitoring approach for marine plastic pollution. In this context, the present study aims to identify molecular and cellular toxicological effects in different *in vitro* and *in vivo* models after exposure to marine and beach plastic samples. For this purpose, the samples were cleaned and extracted, the polymers were chemically characterised, and their extracts were analysed by different types of bioassays. For the detection of endocrine disruption responses in nuclear hormone receptors we used estrogenic (ER), androgenic (AR), pregnane X (PXR), and thyroid (TR) CALUX® bioassays. For the detection of aryl hydrocarbon receptor-mediated activity and detection of genotoxicity, the DR CALUX® and P53 CALUX® bioassays were used, respectively. Ongoing fractionation is performed on extracts that caused mechanism-specific activity in the selected bioassays. Chemical analyses on the bioassay active fractions are used for the identification of toxic compounds. In addition, for the most toxic fractions, we intended to apply high-content screening by Cell Painting assay and different *in vivo* tests including developmental, behavioural, and gene expression analyses using zebrafish embryos (*Danio rerio*).

This study demonstrates the importance of an integrated study design composed of chemical identification of various organic compounds and different *in vitro* to *in vivo* bioassays and Cell Painting, to investigate the toxic effects of micro- and nanoplastics at different trophic levels.

#### **1.09.P-Mo061 Polymer-Specific Biofouling Determines the Vertical Movement of Plastics in Freshwater**

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To improve successful plastic remediation and removal attempts, there is an urgent need to better understand the vertical transport of plastic particles and all factors that affect this transport, particularly in freshwater environments. The density of the particles will determine if a plastic particle stays afloat, sinks, or shows oscillating behaviour. The growth of biological entities (referred to as biofilm formation or biofouling) on the plastic particles can affect the total density of these particles and thus influence the sinking behaviour of plastics. As most research has focused on the marine environment, the effect of biofouling on the sinking behaviour in freshwater is still mainly unknown. The current research studied biofilm formation over time and in function of the polymer type to gain insight into the polymer-specific fate and transport of plastic debris in the freshwater environment. First, a mesocosm experiment simulating a freshwater environment was set up to study the difference in biofilm growth on plastic plates of different polymer types. Second, the biofilm growth on various plastic particles was studied for 62 days (nine weeks) with intermittent sampling. Based on these experiments, the effect of biofouling on the settling onset time and sinking velocity was modelled for various sizes and types of plastic particles. It was indeed proven that biofouling is a polymer-specific process whereby biofilms on polyethylene terephthalate (PET) and polystyrene (PS) showed exponential growth over time. Results of the polymer-specific modelling of the settling onset time indicated that the settling onset time is dependent on both the size and the density of the polymer particle. Smaller particles need less time to start settling compared to larger particles. Moreover, denser polymer types tend to start settling sooner compared to less dense polymer types. For sinking polymer types, the growth of a biofilm is believed to affect the terminal sinking velocity of a particle. The sinking velocity of PET does not seem to change a lot over time and experiences little to no effect of biofilm growth, in contrast to the biofilm formation on PS particles that does seem to have a bigger effect on the sinking velocity. The results of this study help to better understand the vertical transport of plastic particles and assess their fate in freshwater ecosystems.

#### **1.09.P-Mo062 Microplastics on Beaches Along the Coast of Valencia (East of Spain)**

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The contamination of the marine environment by plastics is considered a serious public health issue worldwide and has attracted the interest of the scientific community during the last decade. Plastic fragments with size between 1 µm and 5 mm are known as microplastics (MPs) and their occurrence in environmental samples is widely studied. There are two categories of MPs according to their production, namely the primary MPs that are used in cosmetic products, detergents, etc., and the secondary MPs that are a result of the environmental degradation of bigger plastics. The Mediterranean Sea has become one of the most plastic polluted seas. The presence of MPs in sand samples from seven different beaches of the Valencian community in Spain was studied. The beaches were chosen for their touristic and economic importance. Two different sampling campaigns took place, the first during winter (February 2022) and the second during summer (July 2022) to compare the results and understand the influence of the high touristic activity that usually begins in May. The extraction of MPs from the sand was performed by density separation (floatation) to eliminate heavier (inorganic) particles from lighter (organic) particles. After filtration, the polymer type, color, size, and number of MPs found was performed by stereomicroscopy and micro-Fourier transform infrared (µ-FTIR) microscopy. The contamination of the sand samples by MPs was evident during both winter and summer season but the presence of MPs in the samples from the July campaign was significantly higher because of the increase of anthropogenic activity in the coastal areas due

to the heat. Textile and nylon fibers were the most abundant type of MP with swimming equipment and fishing net being their most probable sources.

#### **1.09.P-Mo063 Biofragmentation of Microplastic in the Process of Digestion by the Snail *Lissachatina fulica* (Bowdich) Tae-Yang Lee, Sang A Kim, Jin Il Kwak and Youn-Joo An, Konkuk University, Korea, Republic of (South)**

Microplastics can be fragmented through various factors in the environment. This study explored microplastic biofragmentation coming out in the digestive tracts of the African giant snail and whether mechanical fragmentation was by the radula. After ingesting polyethylene microplastics, the feces of snails were sampled and watched for identifying biofragmentation. Additionally, the radulas were observed to identify that the biofragmentation of microplastics was not caused by mechanical fragmentation. The surface of microplastics and radula were observed using field-emission scanning electron microscopy (FE-SEM). In addition, energy dispersive X-ray (EDX) point analysis checked the signal of the fragmented particles on the microplastic surface. As a result, the EDX signal of the fragmented particles from egested microplastics changed, indicating that oxidation of microplastics occurred. However, the fragmented particles were not located on the radula, so mechanical fragmentation was not presented. Our findings identified that organisms living in the soil play an important role in the fate of microplastics in the soil.

#### **1.09.P-Mo064 Time-Course Accumulation in Target Tissues and Depuration Dynamics of Polystyrene Microplastics in Mussels and Polychaetes**

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Microplastics (MPs) occupy the same size fraction as sediment particles and some planktonic organisms, presenting a potential bioavailability to a broad scope of biota. They appear on surface waters but are also accumulated in sediments affecting benthic species. Microplastics can be eliminated through feces or accumulated in tissues leading to trophic transfer along the food chains reaching humans through consumption. The time established by FAO for mussel depuration for human consumption may not be effective for the elimination of MPs. The aim was (1) to decipher uptake, accumulation, and localisation of PS MPs in target tissues of *Mytilus galloprovincialis* and *Hediste diversicolor* exposed to various concentrations (103 and 105 MP/mL, LC and HC) of MPs along a time course (1 h, 4h, 24 h, and 72 h); and (2) to study the depuration dynamics (24 h, 48 h, and 7 d) in mussels exposed for 24 h to MPs (104 MP/mL) of different size (0.25 µm, 1 µm, and 10 µm). Mussels from Plentzia (2021) and polychaetes from Galicia (2020) were exposed to MPs, digested to quantify MPs incorporated, and processed for microscopical analysis. Microplastics were found in both species, mainly after exposure to HC. A decreasing trend with time regarding MPs number in mussels was observed, being the opposite for polychaetes and sediments. In mussels, particles were found in the stomach, digestive diverticula, ducts (lumen, epithelium) and gills (only in HC), showing a nongradual decrease over time. The stomach was the unique digestive structure that accumulated MPs after 72 h of exposure to both doses. After depuration, MPs were quantified at all timepoints for sizes of 1 µm and 10 µm, but not for 0.25 µm. Significantly higher quantities of 10 µm MPs than 1 µm MPs were quantified throughout depuration. A significant decrease was observed after 7 d depuration in mussels exposed to 10 µm. Microplastics were found in stomach, intestine, secondary ducts (lumen, epithelium), and gills of mussels exposed to both MPs. A trend to decrease MP number with depuration time in the digestive tract of mussels was recorded for 1 µm and 10 µm, although were still present at longer depuration times (7 d), which exceed the depuration time stipulated for molluscan consumption. Results enabled us to understand the distribution of MPs in tissues of marine species and the depuration dynamics in mussels (helpful for the establishment of depuration protocols for human consumption).

#### **1.09.P-Mo065 Determination of Microplastics in Soil and Water from a Managed Aquifer Recharge System with Secondary Wastewater Treatment Plant Receiving Waters**

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Plastic materials are extremely versatile due to their low density, low thermal and electric conductivity, and corrosion resistance, allowing them to serve as a water and oxygen barrier. Their low price also contributes to their easy and widespread manufacture, where they are used in a wide range of applications from food packaging to medical and technological applications.

Environmental conditions such as solar radiation, abrasion, and diverse interactions with organisms, among many others, cause plastic to degrade and fragment into smaller particles commonly known as microplastics (MPs). Microplastics might accumulate in the natural environment, considering the low degradability of most plastics; thus their analysis and determination are of utmost importance. Wastewater treatment plants (WWTPs) have been identified as one of the highest sources of plastic release into the environment.

In the present work, a simple analytical method for the simultaneous determination of MPs, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), and polyethylene terephthalate (PET), in water and soil samples from a pilot managed aquifer recharge (MAR) system receiving water from a WWTP (Palamós, Spain), has been optimised and validated. Retention and removal of MPs will be assessed in different reactive barriers installed in the system, and the presence of MPs along the course of the WWTP will also be addressed. The method is based on a density separation of the MPs using a salt-saturated floatability solution, followed by an oxidative treatment using hydrogen peroxide and Fenton's reagent, for water and

soil samples, respectively, to eliminate the organic matter present in the samples. After the oxidation, the solution is allowed to settle, before collecting the supernatant, which is filtered through a 1 x 1 cm<sup>2</sup> silicon filter. Once particles are retained onto the filter, it is analysed by FTIR imaging, using a Nicolet™ iN™ 10 MX infrared imaging microscope. The method allowed MP's correct characterisation, and thus was applied to evaluate the removal and retention efficiency of the WWTP as well as the reactive barriers installed. On both, notorious removal of bigger MPs is achieved but still, certain inefficiency on smaller MP's retention was observed, which might lead to the further spread of plastic debris into the aquatic environment. Best options will be proposed, with the aim of further diminishing plastic release into the environment.

#### **1.09.P-Mo066 Impacts of Polyethylene Terephthalate in Manila Clam *Venerupis philippinarum*: Filtration Rate and the Body Accumulation**

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Plastic pollution in the marine environment is ubiquitous and is an emerging problem nowadays. These plastic wastes, especially microplastics (MPs) with very small sizes, can enter the marine environment and sink to the bottom of the sea. The accumulation of MPs in the benthic environment can cause various adverse effects in benthos like crabs, clams, and many invertebrates. In this study, we tried to determine the toxicity and implication of polyethylene terephthalate (PET) MPs on the benthic organism, *Venerupis philippinarum*, and determined the body accumulation of MPs. *V. philippinarum* was exposed to PET MPs with various sizes and shape (diameter <20 µm, Fragment S; diameter 45 µm–75 µm, Fragment M; diameter >150 µm, Fragment L; length 200 µm–400 µm, Fiber S; length > 3 mm, Fiber L) in the range of 0.0005 mg/L to 100 mg/L for 96 h. To determine the impact of PET MPs on the filtration ability of clams, their filtration rates were measured at 0.5 h, 1 h, 2 h, and 4 h after exposure. Additionally, the MP accumulation in the body was observed under microscope. As a result, the filtration rates of clams exposed to every PET MP significantly decreased compared to control organisms and we could determine the accumulation of MPs in the body of clams, especially in the digestive gland. This study can suggest the possibilities of the accumulation of MPs in the marine organisms that live in the benthic environment and used as food resources and also the probabilities of the self-purification disorders in the marine ecosystem.

#### **1.09.P-Mo067 Combining a Novel Radiolabelling Approach and a Traditional Radiometric Technique to Characterise the Biokinetics of Ultralow Levels of Nanoplastics in Mussels**

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Up until recently, only a few studies have addressed the effects of nanoplastics (NPs) on marine biota, mainly owing to the analytical challenges associated with the tracking of ultrafine particles in organisms. This highlighted a need for developing an innovative approach that could help better understand the associated biokinetic mechanisms and their role as vector of transport for other hazardous substances of concern.

In this study, a novel radiolabelling technique was applied to produce two different sizes of NPs (<sup>14</sup>C-polystyrene; 20 nm and 250 nm in diameter). Mussels (*Mytilus* spp.) were exposed to a known concentration (<1 µg/L) of <sup>14</sup>C-NPs for up to 48 h, following by a 14-d depuration period. Bioaccumulation was verified by autoradiography and the levels of <sup>14</sup>C (i.e., NPs) in water and mussel samples were quantified by liquid scintillation counting (LSC). A validated oxidative method was used to prepare mussel samples prior to LSC.

The observations and results obtained from these exposure experimentations and analysis will be discussed in this presentation.

#### **1.09.P-Mo068 Ingestion of SMPs (Small Microplastics < 100 µm) and TWPs (Tire Wear Particles) in King Prawns *Procambarus clarkii* from Highway Stormwater Runoff**

**Beatrice Rosso<sup>1</sup>, Silvia Spinello<sup>2</sup>, Elena Gregoris<sup>3</sup>, Carlo Barbante<sup>3</sup>, Andrea Gambaro<sup>1</sup> and Fabiana Corami<sup>3</sup>,** (1)Department of Environmental Sciences, Informatics, and Statistics, Ca' Foscari University of Venice, Italy, (2)Ca' Foscari University of Venice, Italy, (3)National Research Council (CNR), Institute of Polar Science (ISP), Italy

Plastic pollution from highway stormwater runoff is a direct pathway for conveying plastic and microplastics (MPs) from land-based sources to the aquatic and terrestrial environment. Vehicular traffic mainly (road dust) and atmospheric aerosol are relevant sources of MPs in stormwater runoff. Tire wear particles are the primary source of MPs in road and urban dust. Stormwater runoff, generated by rainfall events, can carry pollutants and MPs in freshwater bodies; they can be dispersed or released, posing a threat to a wide range of freshwater, terrestrial, and marine organisms. Organisms at the bottom of the trophic web (e.g., invertebrates) can ingest particles according to the dimension of their mouthparts, including small microplastics (MPs <100 µm; SMPs) and TWPs. Once in the trophic web, SMPs and TWPs may be bioaccumulated or biomagnified, reaching the top of the food web, even human beings. Due to the benthic lifestyle and diverse feeding strategies, crustaceans have been widely used as bioindicators of environmental quality regarding different pollutants. However, only a few studies specifically examine the presence of SMPs and TWPs in these natural populations, and assessing their baseline levels is still lacking for these species worldwide. There are currently no standardized methods and harmonized techniques for their collection, pretreatments, contamination procedures, and analysis in the biota compartment. Among the widespread invertebrates, king prawns (*Procambarus clarkii*) is a species of cambarid crayfish native to freshwater bodies of Mexico and the United States; it is often an invasive pest introduced in other continents, including Europe. Hence, this research aims to quantify and simultaneously identify

SMPs and TWP ingested by organisms of *Procambarus clarkii* collected in the highway stormwater runoff near Venice, Italy. For each pool, gastrointestinal apparatus, muscle tissues, and the head were dissected and pseudodigested, avoiding denaturation/degradation of these contaminants. The quantification and chemical identification of SMPs and TWPs were performed through a cross-validation technique via Micro-FTIR and Pyr-GC/MS. Different polymer typologies were associated with highway tire degradation (e.g., rubbers, nylon). Pyr-GC/MS confirmed the presence of TWPs using specific markers. A potential translocation among the organism's organs was investigated by comparing the abundance of SMPs and TWPs in the different dissected parts.

#### 1.09.P-Mo069 Bioaccumulation of Plastic Additives and Organic Pollutants in Zebrafish Through Microplastics

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Plastic pollution is an emerging threat with serious consequences for animal health and the environment. Among them, microplastics (MPLs) with a size below 5 mm are the ones that could cause harmful effects to biota since they can be ingested by a wide variety of species. The risks associated with these small fragments come from the material itself and the chemical contaminants that are absorbed into it from the surrounding water. To assess bioaccumulation in tissues, a feeding study of four treatments was conducted with zebrafish for 60 d. Exposure experiments were carried out through the diet (10% of total) and two more experiments, one using clean pellets from a factory and a blank control experiment without MPLs in the fish diet. The analysis of chemical pollutants was by liquid chromatography coupled to high-resolution mass spectrometry (LC- HRMS).

Our results verify the bioaccumulation of chemical pollutants in zebrafish tissues, also over the time. In addition, in some cases, pollutants have more tendency to adsorb to microplastics instead of being desorbed. The family of plasticizers show most of the compounds in level 2 of identification, while plastic synthesizers were quantified as the highest concentration in zebrafish tissues, followed by plasticizers.

Our main findings support the hypothesis that, in this real scenario, plastic additives and chemical pollutants adsorbed on environmental microplastics (EMPLs) bioaccumulate in the fish tissues due to long-term ingestion of MPLs.

#### 1.09.P-Mo070 Small Microplastics (<100 µm) and Nanoplastics in the Venice Lagoon

**Fabiana Corami<sup>1,2</sup>, Beatrice Rosso<sup>1,3</sup>, Giulia Zaia<sup>2</sup>, Anna Mercedi<sup>4</sup>, Lucio Litti<sup>4</sup>, Moreno Meneghetti<sup>4</sup>, Giulia Vitale<sup>3,5</sup>, Carlo Barbante<sup>1,2</sup> and Andrea Gambaro<sup>1,3</sup>, (1)National Research Council (CNR), Institute of Polar Science (ISP), Italy, (2)Department of Environmental Sciences, Informatics, and Statistics, Department of Environmental Sciences, Informatics, and Statistics, Ca' Foscari University, Venice, Italy, (3)Department of Environmental Sciences, Informatics, and Statistics, Ca' Foscari University of Venice, Italy, (4)Department of Chemical Sciences, Nanostructures & Optics Laboratory, Department of Chemical Sciences, University of Padova, Padua, Italy, (5)Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Italy**

Concerning its ubiquity, plastic is considered the most impactful pollutant, which can be hazardous and toxic in relation to the presence of plastic additives (i.e., plasticizers, antioxidant and antistatic agents, lubricants, pigments, dyes, etc.). Plastic objects in the environment can fragment into different shapes, forming macro-, meso-, and microplastics. When forming plastic objects, plastic additives can be mixed with polymers or added later. Hence, plastic additives can be exposed or leach from fragments when breaking; fragment size is important because the smaller the particles, the easier they can be ingested by biota and enter the food web. Compared to large microplastics (>>100 µm, MPs), MPs <100 µm (SMPs) were slightly researched in the environment, as much as nanoplastics in the environment were less explored.

In two sites in the North basin of the Venice Lagoon, Sant'Erasmo and Palude Maggiore, two sampling campaigns were carried out in fall through winter 2019 and summer 2020, before and after the pandemic. Seawater samples were investigated for SMPs and other microlitter components, i.e., plastic additives, plasticizers, and natural and nonplastic synthetic fibers. After oleoextraction and purification, at least three aliquots of seawater samples were filtered on ANODISC filters according to a pretreatment procedure previously developed. The filters were analyzed via Micro-FTIR, where identification and quantification via microscopic counting were performed simultaneously.

Since Micro-FTIR is a nondestructive technique and its imaging LOD is 5 µm, the very same filter was then analyzed via Micro Raman to investigate the presence of plastics particles <5 µm and nanoplastics.

#### 1.09.P-Mo071 Small Microplastics (<100 µm), and other Microlitter Components in the Channels of the Historical Center of Venice: Rio Marin as a Case Study.

**Fabiana Corami<sup>1,2</sup>, Beatrice Rosso<sup>1,3</sup>, Giulia Vitale<sup>3,4</sup>, Elena Gregoris<sup>1,2</sup>, Carlo Barbante<sup>1,2</sup> and Andrea Gambaro<sup>1,3</sup>, (1)National Research Council (CNR), Institute of Polar Science (ISP), Italy, (2)Department of Environmental Sciences, Informatics, and Statistics, Department of Environmental Sciences, Informatics, and Statistics, Ca' Foscari University, Venice, Italy, (3)Department of Environmental Sciences, Informatics, and Statistics, Ca' Foscari University of Venice, Italy, (4)Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Italy**

Microplastic contamination has been an emerging issue in the last decade, although plastic contamination has been known since the 1970s and the term microplastics was coined in 2004.

Much data were produced to assess the distribution of microplastics in different environmental matrices; however, comparing results from different studies is still challenging due to the lack of standardized methods for sample pretreatment and analysis. Several studies employed microscopic methods, which do not allow the identification of polymers, while in other studies, after the microscopic counting, only a subset of particles was analysed via Fourier transform infrared (FTIR). Large microplastics ( $>100\text{ }\mu\text{m}$ , MPs) were widely studied, while small microplastics ( $<100\text{ }\mu\text{m}$ , SMPs) were overlooked, especially in seawater, due to the employment of manta trawls (mesh size  $330\text{ }\mu\text{m}$  or  $100\text{ }\mu\text{m}$ ). Small microplastics (SMPs) can be ingested by invertebrates at the bottom of the food web and enter it with potential bioaccumulation and biomagnification, posing a threat to human beings, as well. Besides, plastic objects have plastic additives, plasticizers, pigments, dyes, etc., which can be added together with the polymer when forming the plastic objects, or being different layers of the objects. Different particle shapes can originate from the fragmentation of plastic objects, which can be dispersed in the environment.

Plastic additives may exert toxic effects on biota once ingested as much as MPs, especially SMPs, may give rise to pseudosatiety phenomena, accumulating in the digestive tract or transferring to other vital organs, also giving rise to inflammatory processes.

In this study, seawater and sediment samples collected from a channel in the historical center of Venice were investigated for the occurrence of SMPs and other microlitter components. This channel, Rio Marin, begins in the Grand Canal, eventually joining the San Giacomo dell'Orto Canal, which also flows into the Grand Canal. Small microplastics and other microlitter components were extracted simultaneously, as much as they were simultaneously identified and quantified via microscopic counting. Samples were assessed using a previously developed pretreatment method and analyzed via Micro-FTIR.

#### **1.09.P-Mo072 Quantification and Characterization of Microplastics and Additives in Soft Tissues of Farmed *Mytilus galloprovincialis* by Micro-FTIR**

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The documented presence of microplastics in edible marine organisms threatens human health through their consumption. In particular, bivalve mollusks are of great concern due to their known ability to filter large volumes of water, including pollutants such as microplastics. Nevertheless, farmed mollusks are still not widely studied, despite farming practices involving the use of synthetic nets, floats, and ropes, which could become a constant source of microplastics for organisms and the environment. This study assesses, for the first time, the presence of small microplastics ( $<100\text{ }\mu\text{m}$ ), additives, and other components, in samples of *Mytilus galloprovincialis* collected from a mussel farm located in the Mar Piccolo of Taranto (southern Italy). The particles were investigated by differentiating in the gastrointestinal tract the gills, and the mantle to understand thoroughly where microplastics are absorbed and accumulated. The pretreatment method is based on pseudo-digestion at temperatures  $<40^{\circ}\text{C}$ , without strong acids or oxidants that can degrade or denature polymers and may underestimate the effective abundance of particles. Polymer identification is important to predict microplastic behaviour in the environment and to evaluate specific analytical strategies. Therefore, a sample purification procedure allowed the simultaneous extraction of all particles and their subsequent chemical characterization by micro-Fourier transform infrared spectroscopy (micro-FTIR) with high yield. Large amounts of synthetic polymers, such as polypropylene, polystyrene, polyamide, and polyethylene, were found by analysing all soft tissues. The predominant polymer was polypropylene, whose source could be linked to the materials used during mussel farming. The acquisitions by micro-FTIR have detected the presence of additives specific to some polymers, such as nylon and bioplastics, but also ultraviolet stabilisers, plasticisers, and dispersant agents in paints for boats, representing an additional threat to the organisms. The particles detected in the mussels suggest a potential pathway for human exposure and highlight the need for continuous monitoring to perform a quantitative risk assessment and to take management measures.

#### **1.09.P-Mo073 Microplastics Contamination of Mussels and Water from the Port of Sines, Portugal**

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Seaports are particularly susceptible to microplastic (MP) pollution through a series of human activities occurring in these areas. Thus, monitoring the biological communities and surface waters is important to assess the impacts of MP pollution. We aim to quantify on a seasonal basis the extent of MP pollution in the Port of Sines, an open deep-water seaport which is located on the southwest coast of continental Portugal and is the main port in the Iberian-Atlantic coast. With relevant national and international importance due to its strategic location and natural characteristics, it offers unique facilities to receive any type of vessel, which makes this port a potential source or sink of contaminants, including MP. Adjacent to this port, there are moderate to very exposed marine areas with regional and national importance for fisheries, tourism, and conservation. The present study reports the quantity, shape, colour, and polymer type of MP in whole soft tissues of mussels (*Mytilus* spp.) and seawater samples, both collected in May 2022. Here, a baseline evaluation of MP concentration at the seawater surface and column was conducted in triplicate with a manta and bongo net ( $150\text{ }\mu\text{m}$  mesh), respectively, for horizontal and vertical tows, in a total of six sampling sites outside and inside the port. Mussels and water were collected in the same sampling sites, stored in cooling boxes, transported to the laboratory, and kept at  $-20^{\circ}\text{C}$  and  $4^{\circ}\text{C}$  with 70% ethanol, respectively, until further processing. Caution was taken regarding



contamination by airborne fibres. Samples were digested with 10% hydrogen peroxide and filtered through a 0.10 µm nylon membrane. Microparticles were classified according to their type into two categories: fibres and fragments. Preliminary results show that all the mussels analysed (n = 60) presented microparticles. Of 311 microparticles observed, 71% were fibres and 29% were fragments. Both categories showed variations in length, size, and colour. Chemical analysis by Fourier transform infrared (FTIR) spectroscopy is in progress. This work will contribute to baseline data regarding microplastic pollution and accumulation in biota, for the Port of Sines and adjacent coastal areas. This will provide a solid background for future research to assess effects of MP in the marine environment and their potential hazards, helping policymakers to make knowledgeable decisions about plastic litter management.

#### **1.09.P-Mo075 Ecotoxicological Assessment of Campania Rivers: A Focus on Microplastic Impact**

**Sara Accardo**, *University Parthenope*

Micropastics (MPs) in freshwater environments have been recognized as one of the important sources of plastic contamination in marine ecosystems.

Sediment is commonly thought of as the “final settling tank” for MPs. However, their impact on the ecosystem and potential ecotoxicological effects are still unclear.

This study presents the first investigation on the abundance and distribution of MPs contamination in freshwater streams in Campania, Italy.

Sediments were collected from different sampling points along the rivers to understand how the microplastic abundance varies along the river flow. Samples were analyzed for MPs content and each MP was visually identified, counted, photographed, and categorised according to size, colour, and morphology. Microplastics were characterised using Fourier transform infrared (FTIR) spectroscopy and Raman.

Moreover, the ecotoxicological status of these rivers were also assessed by performing a battery of ecotoxicological tests with organisms belonging to different trophic levels (bacteria, algae, and crustacean). All results were integrated to obtain a Toxicity Battery Index (TBI). The safety threshold of MPs for protecting the freshwater organism was also determined and used to assess the potential ecological risk posed by MP in the riverine ecosystem of Campania.

In addition we aimed at expanding the knowledge on sublethal effects caused by exposure to sediments containing MPs. Accordingly, chronic effects on *Daphnia magna* were assessed.

Micropastics were identified at all sites in the range of 0.5–3 particles/g. Fibres were the dominant MP types by fragments and sphere. Black particles were the most common ones followed by transparent and coloured MPs. Based on the attenuated total reflectance (ATR)-FTIR analysis, polyethylene, polyethylene terephthalate, polystyrene, and polypropylene were the main plastic polymers. The test battery integrated index did not allow highlighting significant differences among the sites and showed a general slight ecotoxicological risk.

All data suggested that in the Campania rivers, the ecological risk posed by MPs on aquatic organisms was not negligible. These results represent a preliminary assessment of the MPs' impact on rivers and more detailed studies are needed, especially to expand the set of exposure and toxicity data used in the probabilistic risk assessment.

#### **1.09.P-Mo077 Development of an Analytical Method for the Detection of Nanoplastics in Biological Samples: The Case of the Chironomid *Diamesa tonsa***

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In recent years, the scientific interest in nanoplastics (NPs) has increased because their unique properties make them potentially hazardous for organisms. However, limited data on NPs exposure are available due to the lack of reliable methods for their detection in complex matrices. In this perspective, a promising approach to overcome some of the analytical limitations of NPs detection is the use of bioindicators.

*Diamesa tonsa* is a chironomid (Diptera: Chironomidae) with larvae stages living in cold freshwaters, mainly in streams fed by snow and ice. Nanoplastic contamination has been recently demonstrated in surface snow at high altitude in the Alps. The present study aims to investigate the role of *D. tonsa* as a sentinel of NPs pollution. For this purpose, we tested on *D. tonsa* larvae the efficiency of a recently proposed method for the characterization of NPs in biological samples.

*D. tonsa* larvae were sampled in a glacier-fed stream of the Adamello-Brenta National Park (Trento, Italy). To validate the extraction and purification process, samples were spiked with palladium-doped polystyrene nanoplastics (Pd-PS-NPs) and then processed. The protocol involves enzymatic digestion, microfiltrations, centrifugations, and a microspotting on functionalized surfaces followed by Raman analyses. To obtain recovery rates, Pd concentrations were assessed in all extraction steps by inductively coupled plasma-mass spectrometry (ICP-MS) analyses.

Preliminary results showed a recovery of 40% in the final samples as measured by ICP-MS analysis. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Raman evidenced that the enzymatic approach did not cause

alterations in Pd-PS-NPs. They also confirmed the effective presence of Pd-PS-NPs in the final samples allowing a semiquantitative characterisation.

The method enables the detection and semiquantitative analysis of NPs in a complex matrix; the evaluation of the presence of environmental NPs in *D. tonsa* is under study. These preliminary findings are essential to develop analytical techniques and approaches for the detection of NPs in the environment, to gain key data for NPs environmental risk assessment.

#### **1.09.P-Mo078 Microcosm Study of the Effects of Polyester Microfibers on Indigenous Marine Amphipod (*Cyphocaris challengerii*) in the Strait of Georgia**

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Microplastics remain contaminants of great concern because of their abundance, ubiquitous in all habitats of the world and threats posed to organisms inhabiting these habitats. While polyester (PET) microfiber is the most abundant microfiber found in Indigenous marine zooplankton, little is known about its effect on marine amphipods. We investigated the effects of PET microfiber on the survival, microfiber ingestion and retention, predation, and fecal pellets (FP) of marine amphipod (*Cyphocaris challengerii*) at different concentrations (0, 10, 100, 1000, 10 000, and 50 000 Mf/L) and exposure times (24 h, 48 h, and 72 h) using microcosm experiments. Our study demonstrated that microfiber concentration had a greater influence on parameters investigated than exposure time. The exposure of *C. challengerii* to PET microfiber did not affect their survival. However, it significantly affected their feeding with a reduction in predation rate as microfiber concentration increased. This would make *C. challengerii* transfer less energy to their predators in the Strait of Georgia (SoG). The average number of ingested microfiber and ingestion rate increased significantly with microfiber concentrations. Nonetheless, microfiber retained was extremely low ( $0.037 \pm 0.06$  per ind<sup>-1</sup> h<sup>-1</sup>) implying that most *C. challengerii* evacuated ingested microfiber within 48 h postexposure. Also, encapsulation of PET microfiber in FP significantly increased their sinking velocity and density, and this will practically promote the downward transport of carbon in the SoG. Hence, there is an urgent need for a drastic reduction of the release of fibers from laundry and the design of equipment that will efficiently trap fibers and other microplastics types from laundry effluents in the wastewater treatment plants before their release into the environment.

#### **1.09.P-Mo079 Exploring Changes in the Metabolome of Freshwater Benthic Invertebrates Exposed to Polyethylene Microplastics: A Two-Generational Investigation**

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Constructed wetlands are primarily built to reduce pollutant loads in treated wastewater or stormwater before they are released into rivers and streams. However, they can also become sinks for retained pollutants that accumulate over time. A recent study found the average abundance of microplastics in the sediment of constructed wetlands in Australia was 1050 particles/kg dry sediment, which is higher than 80% of reported freshwater sediment samples globally. In addition, polyethylene fragments were the dominant microplastics identified. Consequently, it is important to understand the potential effects of microplastics on wetland benthic organisms. We therefore investigated the effects of irregular shaped polyethylene microplastics (1 µm –45 µm) in sediment on a common freshwater benthic invertebrate, *Chironomus tepperi*, at four environmentally relevant concentrations (125, 250, 500, and 1000 microplastics/kg sediment) over two generations. In the parental generation, significant reductions were observed for survival and emergence at both 500 and 1000 microplastics/kg compared to the control group, whereas growth (body length and body mass) was only affected at the highest exposure concentration. Conversely, no significant differences were observed for growth, survival, or emergence between treatments for the subsequent generation. To further understand the effects associated with sublethal exposure to microplastics, the metabolite profiles of *C. tepperi* larvae were also investigated. A dose-dependent decrease in several amino acids was observed in parental generation, which likely reflects a physical disruption of food/nutrient acquisition, or potentially an impact on energy generation. These results demonstrate the application of integrated apical and metabolomics approaches to provide useful information about the potential organismal and physiological effects of polyethylene microplastics on a benthic invertebrate under environmentally relevant concentrations.

#### **1.09.P-Mo080 Interaction of Marine Algae and Nanoplastics: Impact on Growth and Extracellular Polymeric Substances (EPS) Production**

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Plastic pollution is one of today's most visible environmental problems, and its many aspects have been widely discussed in the media. However, the impact of plastics on aquatic and terrestrial ecosystems, as well as on human health, still remain largely unclear. Especially regarding nanoplastics (NPs, dimensions <1 µm), a knowledge gap exists. Nevertheless, it is assumed that the exposure route, extent and rate of biouptake and bioaccumulation, and the nature of adverse effects will differ from those of microplastics, due to the reactivity features of nanoscale entities and the fact that they are small enough to cross biological barriers.

The aim of the study was to look at the impact of nanoplastics on the growth cycle of marine phytoplankton species, and the effect on the production of extracellular polymeric substances (EPS) of the phytoplankton. Extracellular polymeric substances were analyzed as a proxy for aggregate formation as their sticky properties can induce heteroaggregation, and it is a known stress-response.

The algae species used for the experiment was *Rhodomonas salina*, a relevant algal species for the North Sea food web. The assumed realistic concentrations of nanoplastics are derived from microplastic concentration data in the North Sea and the conversion factor of  $10^{14}$  as proposed by Besseling et al., 2019. This factor is based on mass conservation principles, for the fragmentation of spherical particles with a size of  $>0.1$  mm–5 mm into 100 nm particles. The nanoplastics used are fragmented aged polyethylene terephthalate (PET,  $d = 0.68$   $\mu\text{m}$ ,  $D_{90} = 1.0$   $\mu\text{m}$ ) and fragmented polypropylene (PP,  $d = 1.7$   $\mu\text{m}$ ,  $D_{90} = 2.9$   $\mu\text{m}$ ), produced by the Joint Research Centre (JRC) of the European Commission in Milano.

We observed a significantly lower total cell yield at the end of the experiment after exposure to both aged PET and PP. For PP, the total cell yield decreased with increasing NP concentration. For PET, the total cell yield increased with increasing NP concentration, but was always lower than the cell yield of the control group. Also, an increase in EPS production is observed. This research gives valuable insights on the increased EPS production and possible aggregate formation after exposure to NPs. This can both affect the density and thus the location of the algae in the water column, as the availability of the algae to primary consumers, as their size increases. It also affects the stability, and thus the fate and transport of the nanoplastics in the water column.

#### **1.09.P-Mo081 Influence of Particle Size on the Trophic Transfer and Uptake of Eu-PS by the Terrestrial Snail: *Cantareus aspersus***

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There is a significant concern about the potential toxicity of nano- and microplastics (NMPs) in plants and organisms. Due to their small size, these NMPs might end up in organs and tissues and transfer throughout food chains. Moreover, there is a lack of knowledge on the fate of these NMPs on soil biota. To understand the influence of particle size on the trophic transfer of NMPs in a specific food chain, their uptake in plants (*Lactuca sativa*) and their transfer to garden snails (*Cantareus aspersus*) are investigated. Two particle sizes were tested: 100 nm and 300 nm of Europium doped polystyrene (Eu-PS). Plants were hydroponically grown in three different concentrations (low, medium, high: 15  $\mu\text{g/L}$ , 150  $\mu\text{g/L}$ , and 1500  $\mu\text{g/L}$ , respectively) of Eu-PS spiked Hoagland solution for 21 d. Shoots were subsequently fed to garden snails (*Cantareus aspersus*) for 21 d. At the end of the experiment levels of Eu-PS were determined using inductively coupled plasma-mass spectrometry (ICP-MS) for the Hoagland solution, lettuce (shoots and roots), and of the snails' organs (soft tissue, digestive gland, and kidney). No leaching from the Eu-PS particles was detected in this study. Europium was detected in the roots of the plants in all exposure concentrations, and for both sizes, whereas for the shoots, Eu was detected only in the medium and high concentrations. The Eu-PS of both sizes was not detected in the digestive glands or in the different dissected organs of the snails. However, Eu was detected in the feces of those exposed to lettuce shoots of medium and high Eu-PS concentrations. These results indicate that snails did not accumulate Eu-PS in the organs and that NMPs pass through their system and end up in their excrement. This study shows, for the first time, the uptake and transfer of NMPs of 100 nm and 300 nm in the plants. In addition, our results show the transfer of the NMPs into the food chain with no observed accumulation in the snails. This developed approach can be used to assess the trophic transfer in various food webs leading to a better understanding of the fate of NMPs in the environment.

#### **1.09.P-Mo082 Bad Romance — The Relationship Between Microplastic and Filter Feeders**

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Plastic pollution is a growing threat to marine organisms and ecosystems. Once the plastic litter reaches the ocean it is prone to degradation due to forces such as UV, water turbulence, heat, and biological activity. This increases the particle's surface roughness and porosity which causes the decrease in particle size to micro-sized particles (MP). As the size of the particles decreases the range of organisms able to ingest the particles increases. Microplastics are often transported from the water column to marine food webs by filter-feeder organisms which play an important role in benthic-pelagic coupling. The ingested MPs may accumulate in the gut or may pass through the digestive system where they are subject to different pH fluctuations and to various digestive enzymes, ultimately excreted within the organism's feces. Feces sink down the water column transporting carbon to deeper waters, therefore acting as a vector of MP through marine zones. Ascidiaceans (Chordata, Ascidiaceae) are efficient filter feeders, able to remove minute particulate matter from the water column. Therefore, they present fundamental opportunities for understanding the interaction of plastic debris with marine organisms. Here, we use engineered MP particles of high environmental relevancy to examine the consequences of transit through an organism's digestive system. We examined the changes of the particle's surface area, size, morphology, and surface functionality. Particles were fed and later extracted from the feces of two solitary ascidian species: *Styela plicata* and *Polycarpa mytiligera*. Additionally, we measure the effect of the MP on gametes quality, fertilization, and early development of the ascidian *Hermania momus*. Our preliminary results reveal that the extracted MP (following digestion by ascidians) had a rough surface area coated by organic matter. This change in surface area and functionality may later influence the MP availability to other organisms. Currently, we examine how ascidians clear MP sized

between 60 µm and 0.45 µm from the water column. Moreover, we expose gametes to MP treatments examining the fertilization success. Reproduction by spawning and external fertilization is very common among invertebrates; in the open water gametes are vulnerable to MP and other pollutants. Overall, as MPs transfer through marine food webs across marine ecosystems, it is essential to promote the understanding of the consequences of biological digestion.

#### **1.09.P-Mo083 Development of Novel Molecular Indicators of Emerging Contaminants in Aquatic Environments**

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Projections estimate that 445.25 million tonnes of plastic will be produced in 2025 and will continue to increase by 30% in 2050. It has been predicted that 79% of plastic produced up to 2015 is residing in landfills and the environment, where it is degrading to micro/nanoparticles and having a negative effect on aquatic species. Bioplastics have been developed as a biodegradable alternative to traditional plastics, but little is known about their effect on aquatic species. Polylactic acid (PLA), a popular biopolymer, has been reported to induce behavioural and biochemical changes in *Danio rerio*, increase reactive oxygen and nitric oxide species in *Physalaemus cuvieri* tadpoles, and cause reduced survival in *Daphnia magna*. Although PLA has a similar/higher sorption capacity to conventional microplastics (MP), little is known about the toxicological mixture effects of bio-MPs and environmental contaminants.

This study aims to investigate the toxicity of PLA, while using nanopolystyrene (NP) as a control particle for the exposures. Mixture effects with emerging contaminants of concern are also investigated to determine if biopolymers exacerbate the toxicity of known pollutants of interest. Three freshwater fish cell lines will be used in this study: PLHC-1, topminnow liver (*Poeciliopsis lucida*); RTG-2, rainbow trout gonad (*Oncorhynchus mykiss*); and ZF4, zebrafish embryo (*Danio rerio*). Cytotoxicity and genotoxicity effects, as well as genomics, are used to evaluate the biological effects at a cellular level. In addition, whole organism comparison studies of the effects of NP on zebrafish embryos will be compared to the ZF4 embryo cell line to evaluate the effects on lower levels of organisation when low levels of mortality in zebrafish embryos were seen after 96 h exposure. These comparisons should provide distinct results of the potential for chronic toxicity effects on aquatic species that are currently unseen in standard acute exposure findings.

#### **1.09.P-Mo084 Sublethal Effects on Behaviour and Feeding of Bioplastic Microparticles in *Daphnia magna***

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Plastic pollution is nowadays an increasing concern environmental problem. In the past few years, in retail stores new bioplastics have been taking over, to offer a greener solution. These bioplastics are attained by renewable feedstock, such as polylactic acid (PLA) and polyhydroxybutyrate (PHB). As their demand is increasing, there is a need to investigate their environmental fingerprint, whilst studies have shown that the toxicity of their plastic additives may be higher than in fossil plastics. This study focused on the toxic effects to aquatic indicator organisms (*Daphnia magna*) of different single use items made of biomaterials PLA and PHB, and a highly used fossil material polyethylene (HDPE), in addition with their analytical standards. Assays were performed both with microparticles (MPs) of each material and their leachates. *D magna* juveniles were exposed for 24 h to different concentrations of MPLs and their leachates and effects on feeding and behaviour measured. Results showed a similar toxicity of PLA and PHB microplastic particles with an EC50 on feeding inhibition of 0.15 g/L. Behavioural defects of 0.15 g/L of bioplastics on baseline locomotion activity, visual motor responses (VMR), maximal response (MAX) to light, and nonassociative attenuation to repetitive light stimuli evidenced similar toxic effects of single-use consumer products made of PHB and PLA biopolymers. Plastic leachates obtained in methanol were about five times less toxic than particles. Particles and leachates of HDPE items and standards were less toxic than those of bioplastics. Overall, our results indicate that bioplastics and their leachates are more toxic than those of HDPE.

#### **1.09.P-Mo085 Ecotoxic Effect of Biodegradable Plastic Bags Leachates on Marine Organisms**

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Plastic pollution is one of the greatest emerging threats to the environment in our time. Plastic can enter marine habitats on different pathways, e.g., as lost material from the fishing and shipping industry as well as migrated from land-based trash.

The combination of biotic and abiotic degradation leads to alternation and fragmentation of plastic and often to the formation of micro- (MP) and nanoplastics (NP).

The threat large and small plastic particles pose to marine life gained more and more attention during recent years and thus the usage of so-called bio- and biodegradable plastic has gained more attention. Bioplastics are promoted as an alternative to conventional petroleum-based nonbiodegradable plastics

However, whether biodegradable plastics are successfully tackling the problem is questionable as laboratory and field studies showed contrasting outcomes. Today, it remains unclear whether biodegradable formulations are advantageous to reduce plastic impact compared to conventional polymers. Plastics contain a complex mixture of known and unknown chemicals, some of which can be toxic. Bioplastics and plant-based materials are marketed as sustainable alternatives to conventional plastics. However, little is known with regard to the chemicals they contain and the safety of these compounds.

In this view the aim of this work is to evaluate the potential ecotoxicity of different types of plastic bag leachates in seawater. Conventional (polyethylene) and compostable bags (Mater-Bi) were cut and placed in artificial sea water for a period of three months. Periodically ecotoxicity tests upon different organisms (*Dunaliella tertiolecta*, *Vibrio fischeri*, and *Paracentrotus lividus*) were carried out. Results showed that *D. tertiolecta* was significantly affected by both kinds of plastic bags already after the first week of leaching to a slightly greater extent with regard to compostable bags. In the case of *P. lividus* the effect became evident starting from the 8th week for the conventional plastic bags and the last week of exposure for conventional ones. Instead, all the leachates exerted a hormetic effect upon the bacteria *V. fischeri*.

#### **1.09.P-Mo086 Role of Container Materials on Polycyclic Aromatic Compound (PAC) Concentrations in Aqueous Phase: Implication for Aquatic Ecotoxicity Determination**

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In historically contaminated sites such as coking plants, other families of organic pollutants than the 16 regulated polycyclic aromatic hydrocarbon (PAHs) can occur. Polar polycyclic aromatic compounds (PACs) are present in the initial pollution and can be generated over time. Their aqueous solubilities are greater than those of the PAHs, and may induce the contamination of groundwater resources. It is important to evaluate the potential risk associated with the occurrence of PACs in bodies of waters.

A classical approach is to run a standard aquatic bioassay using model organisms, as rotifer (*Brachionus calyciflorus*) (ISO 20666, 2009) and algae (*Pseudokirchneriella subcapitata*) (ISO 8692, 2012) to evaluate the associated ecotoxicity.

The aim of this study is to determine the fate of PACs during standard *in vivo* aquatic ecotoxicity testing. Indeed, rotifers and algae tests are usually performed in polystyrene (PS) 24-well–96-well microplates, respectively, and little is known about the PAC sorption capacity to PS under test conditions. However, this information is crucial to determine the actual PAC concentrations to which organisms are exposed.

Tests were performed on a coking plant soil leachate stored in PS and glass bottles. Conditions were determined based on the bioassay conditions, i.e., 48 h at 25°C in darkness for rotifers and 72 h at 22°C under light for algae. Leachates were collected every 24 h for PS containers and until the end for glass bottles.

Results showed that the container material has a strong impact on the measured PAC concentrations in solution, especially for PAHs. A strong decrease in PAH concentrations was observed in PS containers under both test conditions and the effect was most apparent for high molecular weight compounds, most likely due to sorption phenomena. This decrease was absent with glass containers. Also, a reduction in low molecular weight compound concentrations was noticed with both materials in algae test conditions that may result from PAH photodegradation.

Consequently, PAH concentrations to which organisms are exposed during ecotoxicity tests are expected to be much lower than expected based on PAH levels in fresh leachate.

These results pointed out the need to circumvent this experimental bias in order to calculate reliable ecotoxicity parameters. This could be done through an adjustment of protocol to the physicochemical properties and reactivity of studied molecules, or a calculation of theoretical concentrations based on kinetic parameters of the process involved.

#### **1.09.P-Mo087 Impacts of Polyethylene Terephthalate Microfiber and Microfragment on ROS Generation and Enzyme Activity in Manila Clam *Venerupis philippinarum***

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The increasing global contamination of microplastics (MPs) in the marine environment is a significant concern to the environment and public health. The small size of microplastics implies that MPs are readily ingested by marine organisms and cause problems in marine ecosystems. The objective of this work is to identify the biological toxic effects of polyethylene terephthalates (PETs) MPs depending on their shape and size. The marine clam *Venerupis philippinarum* was exposed to the suspension of fragments and fiber-shaped PET MPs with different size (<20 µm, Fragment S; 45 µm–75 µm, Fragment M; >150 µm, Fragment L; 200 µm–400 µm, Fiber S; 3 mm, Fiber L) in the range of 0.0005 mg/L to 100 mg/L. The results showed that to gain insights into the mechanism of PET-induced toxic effects, reactive oxygen species (ROS) contents, the activities of superoxide dismutase (SOD) and acetylcholinesterase (AChE) in the gill and the digestive gland were measured. Both two different types (fragments or fibres) of PET MPs did not cause mortality at the tested concentrations over 96 h. However, the exposure to each different-shaped PET MP significantly increased ROS levels in all tested concentrations compared to control value. Generally, increasing oxidative

stress to ROS also caused increased SOD enzyme activity. The AChE activities in the gill showed significant increase after exposure to fragment-shaped PET MPs. There was no significant difference in AChE activity in the gill and the digestive gland exposed to fiber-shaped PET MPs. This work will provide empirical evidence of the toxicity of PET MPs to better understand the effects of MPs in aquatic organisms.

#### **1.09.P-Mo088 The Effect of Dioctyl Terephthalate on Biofilm Formation of Plastic-Degrading Bacterium *Rhodococcus ruber* on PVC Film**

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Phthalate esters are the most widely used plasticizers and were found to have an annual consumption of over 30 million tons in the year 2017. However, because many phthalates are the cause behind reproduction and developmental toxicity, their use is gradually being phased out. The European Union (EU) has introduced dioctyl terephthalate (DOTP) as a less toxic nonphthalate alternative for the widely used di-(2-ethylhexyl) phthalate (DEHP). Presently, other than studies on humans and rats, there are no data available on the biodegradation and toxicity of DOTP on microbes. Therefore, this study aims to discover the metabolic pathway and toxicity of DOTP upon internalization by *Rhodococcus ruber*. The metabolic pathway was found by analysis of media containing PVC plastic with 0.1% DOTP using an Orbitrap mass spectrometer. Biodegradation of DOTP had similar metabolites which were found in the degradation of DEHP with a final product of benzoic acid. The toxicity was found by determining oxidative stress when exposed to different concentrations of DOTP (1%–10% v/v) in PVC plastic using the CellROX™ Green Reagent and Hoechst 33342. Oxidative stress was apparent even from 1% v/v DOTP in the plastic.

#### **1.09.P-Mo089 Survival and Reproduction Effects of Four Microplastics on *Ceratophysella denticulata*, *Folsomia candida*, *Heteromurus nitidus*, and *Sinella curviseta***

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An estimated 467 kt of soil contamination is caused by agricultural plastics annually. Plastics that end up in the soil break down into secondary microplastics (MPs). Not much is known about the effects these MPs have on soil functioning and organisms residing in the soil. To evaluate these effects and properly assess the risk they pose to the environment, experimental data are needed on the effects MPs have on the survival and reproduction of model organisms. This research aimed to assess the toxicity of four MP types derived from commonly used agricultural plastics to different Collembola species, representing an important and highly diverse class of soil living arthropods. Starch-PBAT blend MPs were produced from both virgin mulching films as well as mulching films that were artificially aged by mechanical recycling. In addition, MPs were made from linear low-density polyethylene (LLDPE) virgin mulching films and low-density polyethylene (LDPE) MPs that underwent the same mechanical recycling process as the starch-PBAT blend MPs. Four Collembola species were used: *Ceratophysella denticulata*, *Folsomia candida*, *Heteromurus nitidus*, and *Sinella curviseta*. The use of these four species ensures the inclusion of epedaphic, euedaphic, and hemiedaphic species, as well as sexually reproducing and parthenogenetic Collembola. Each of the species was exposed using Lufa 2.2 spiked with the MPs at nine concentrations: 0.0016, 0.008, 0.04, 0.2, 1, 2, 3, 4, and 5% (w/w dry soil). Eight controls were included, while all treatments had five replicates. Tests were started with 20 age synchronized (40–43 –d-old) adults of the sexually reproducing species, while for the parthenogenetic *F. candida* 10 adults were used per test jar. All species were exposed for three weeks, except for *C. denticulata*, since the eggs of this species can take up to two weeks to hatch, asking for a four-week exposure period. No significant effects of starch-PBAT blend MPs from virgin PBAT films have been observed in *F. candida* or *S. curviseta* at any of the tested concentrations. The analyses of the samples from this study are ongoing, and results will be presented at the meeting in May 2023.

#### **1.09.P-Mo090 Toxicological Interaction of Microplastic and Polyaromatic Hydrocarbons on Macrophyte**

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The potential effect of microplastic has been investigated in various organisms yet such a study on macrophyte is relatively scarce. Direct contact of plastic particles on plants was found to exert impacts on growth rate primarily due to shading, while the chemicals related to the plastic may also cause negative effects. To elucidate the interactions between toxic chemicals, plastic, and macrophyte, we designed an exposure system containing microplastic, polyaromatic hydrocarbons (PAHs) contaminated sediment, and duckweed *Lemna minor*. This system creates agitation; therefore the contaminated sediment could be suspended in the water column to interact with microplastic and plants. After exposure, the PAHs partitioning between these components will be analyzed. We hypothesize that the microplastic can contribute to the transfer of PAHs from sediment to the plants thereby causing stronger toxic effects. Growth inhibition and relevant sublethal biomarkers including antioxidant capacity/oxidative stress will also be assessed. This study will provide insight on the potential toxic effects of microplastic for both particle and chemical effects on macrophytes.

#### **1.09.P-Mo091 Combined Toxic Effects of Cadmium and Environmental Microplastics in *Aphanius fasciatus* (Pisces, Cyprinodontidae)**

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Microplastics (MPs), plastic particles smaller than 5 mm in diameter, have received extensive attention as new environmental pollutants with still unexplored potential ecological risks. In this study we investigated the effects of cadmium (Cd) and environmental MPs in the marine killifish *Aphanius fasciatus*, both separately and in combination. Female individuals were exposed to Cd and/or MPs for 21 d, and the subsequent effects were monitored by a combination of biochemical, histological, and molecular toxicity markers. Exposure to Cd, but not to MPs, increased metallothionein content and metallothionein gene MTA mRNA levels both in liver and gills. On the contrary, we observed a significant oxidative stress response at histological, enzymatic, and gene expression levels to both toxicants in both tissues, particularly in gills, but not very weak evidence for the interaction between the two effectors. Finally, exposure to both MPs and Cd induced spinal deformities, although bone composition was only altered by the latter, and MTA mRNA bone levels only differed from controls in the doubly exposed samples. Our results indicate a major effect of MPs on gills at different organizational levels, and suggest a possible, if mild, capacity for enhancing the toxic effects of heavy metals.

#### **1.09.P-Mo092 Are Polyethylene and Polystyrene Microparticles Capable to Disrupt the Intestinal Function of Rainbow Trout?**

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Polyethylene (PE) and polystyrene (PS), the main polymer pollutants of the aquatic environment, were tested to achieve information about their potential toxicity for intestinal tracts of freshwater fish. A study was done with rainbow trout (*Oncorhynchus mykiss*) as a model organism, while the PE ( $46.6 \pm 11.3 \mu\text{m}$ ) and PS ( $52.5 \pm 11.5 \mu\text{m}$ ) were submitted to fish in commercial feed pellets for six weeks. Both PE and PS were tested in the same concentration doses – 0.5%, 2%, and 5% of the feed/day. Our study aimed to characterise PE and PS effects on the gut microbiota alterations and gene expression changes in intestinal tissue.

In regard to microbiota, we observed higher diversity of bacteria species in the control group compared to groups exposed to both tested microplastics. Moreover, in the case of PE the amount of bacteria decreased in concentration manner also. The lower diversity of bacteria phyla in gut is a sign of dysbiosis and is associated to outbreak of fish diseases. In our study, the *Firmicutes* and *Fusobacteria* were the most abundant phyla of all; their mutual ratio, however, was affected by the PE and PS exposure. Both these phyla are producers of short-chain fatty acids that regulate the energy metabolism, improve intestinal integrity, and support gut reparation. Analysis of gene expression showed significant changes of carrier family 9 member A1b (*slc9a1b*), ghrelin/obestatin prepropeptide (*ghrl*), and transferrin-a (*tfa*) gene expression mainly in the highest (5%) tested concentration. Both *slc9a1* and *ghrl* interact with the immune system and cytokine release. Moreover, *slc9a1b* is involved in pH regulation and sodium proton antiporter activity, and *ghrl* has an appetite-stimulating effect and induces reactive oxygen species (ROS) production. Additionally, *tfa* is participating in iron ion binding and maintaining of homeostasis and alterations at mRNA expression level may potentially provoke the ROS release in intestine.

With respect to the results obtained so far, it is clear that microplastics submitted orally may affect the function of the digestive tract, immune system, and induce oxidative stress. As plastics in microsizes are known to be a serious threat to the aquatic biota with yet not completely described consequences, these alterations in intestinal function might predict that the negative effects of polymers can be expected at the level of the digestive tract as well.

#### **1.09.P-Mo093 Do Polystyrene Microparticles Affect the Early-Life Stages of Zebrafish (*Danio rerio*)?**

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Plastic waste pollution is considered one of the biggest problems facing our planet, which is caused mainly by an excessive use of this material, its high resistance in the environment and poor waste management. Under mechanical and biological degradation, plastics can be broken down into smaller fragments. Microplastics (<5 mm; MPs) have been discovered in the bodies of various organisms, across all trophic levels of the aquatic environment. Although many studies have focused on the impact of MPs on aquatic organisms, only a small number of them study the effect of MPs on early developmental stages of fish. The tested organism zebrafish (*Danio rerio*) has been widely used as an emerging disease model to investigate human genetics and early-stage development due to its high similarity with the human genome and in addition, zebrafish embryos are transparent, making it possible to reveal a disorder or malformation caused by exposure to MPs.

In our study, the effect of polystyrene microspheres (50  $\mu\text{m}$ ) on zebrafish embryos was tested in three concentrations – 0.01 g/L, 0.1 g/L, and 1 g/L for 96 h with 18 eggs of zebrafish per each concentration. The plates were kept in a temperature-controlled test environment (26°C) with a photoperiod of 12 h light/12 h dark. Testing solutions were replaced every day to make sure that the

test concentrations remained their nominal values. During the test, each plate was checked for coagulation of fertilized eggs, lack of somite formation, lack of detachment of tail-bud from the yolk sac, lack of the heartbeat, hatching rate, and developmental malformations. After 96 h exposure, eight replicates were created from each test group, with each sample containing 10 mg of embryos to subsequent PCR analysis of selected genes and proteomic to the systematic identification and quantification of selected proteins. The significant changes at tested concentrations were compared to the control group, without microplastic addition.

#### **1.09.P-Mo094 Automated Quantification of Fluorescence Signals for Apoptosis and Necrosis in Bivalve Hemocytes (*Mytilus edulis*) using Fiji/ImageJ as a Proxy for *in vitro* Toxicity Assessment**

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Hemocytes are responsible for cell-mediated immunity in bivalve mollusks. Apoptosis and necrosis (cellular death) are part of the integral cellular processes that influence essential physiological functions, including cellular immunity. It has been established that the physicochemical properties of micro- and nanoplastic particles (MPs/NPs) can directly affect cellular uptake mechanisms and immunotoxicity. However, the standardized method for systematic analysis of sensitive cellular-level endpoints induced by MPs/NPs exposure is still lacking. Hence, this study aims to validate and apply a microscopy-based method for evaluating the induction of apoptosis/necrosis in bivalve hemocytes as a proxy for micro- and nanoplastic toxicity assessment. The *in vitro* assay was carried out via a dual staining approach using an apoptosis detection kit (Biovision) and by the complementary application of confocal microscopy and ImageJ, an open-source image processing program. To ensure the validity and sensitivity of the automated quantification of fluorescence signals for viable, apoptotic, and necrotic cells, the output generated by the automated method was compared with manual and flow cytometry-based quantification methods. Results revealed that, with proper optimization of settings, the automated quantification method is a reliable and reproducible method that uses open-source software for image analysis, suitable for large-scale image quantities, and is applicable to evaluate the induction of apoptosis in fluorescent images of various cell types.

#### **1.09.P-Mo095 Toxicity Assessment of Used and Pristine Cigarette Filter Microplastics and Their Leachates to *Daphnia magna***

**Victor Carrasco Navarro, Su'ad Musse and Jussi Kukkonen, University of Eastern Finland, Finland**

Littering is one of the main causes of plastic contamination in the environment and it may cause the production of microplastics (MPs) under several conditions. Microplastics are a very complex group of contaminants where sizes, type of polymer, and additives, among other factors, may influence their toxicity. Cellulose acetate is one polymer widely used in cigarette filters (CF) and one of the most prevalent sources of littering and MPs.

In our study, the toxicity of used and pristine CF microplastics and their leachates was assessed using the water crustacean *Daphnia magna*. *Daphnia* species inhabit very diverse water bodies and are a key prey species of planktivorous fish and other larger invertebrates, which highlights their importance in aquatic ecosystems.

The leachates were prepared by shaking a suspension of CF (80 g L<sup>-1</sup>) in artificial freshwater 2.5 mM in Ca<sup>2+</sup> and Mg<sup>2+</sup> for 15d at 160 rpm. Following that, the suspension was filtered (0.22 µm pore size membranes) and the resulting leachate was considered as pure, 100% leachate. Dilutions to 5, 2.5, 1, 0.5, 0.25, 0.125, and 0.025% were used as the concentration range. Furthermore, we cryogrounded both pristine and used cigarette filters previously cut in smaller pieces (~20 mg) by pestle and mortar after cooling the materials with liquid N<sub>2</sub>. The obtained microplastic particles were used to prepare suspensions at concentrations of 4, 2, 0.8, 0.4, 0.2, 0.1, and 0.02 g L<sup>-1</sup>, equivalent to the concentrations used in the leachate tests.

*Magna* neonates (<24 h old) were added to experimental vessels with 10 ml of the experimental waters at the concentrations mentioned above. Mobility of the organisms was monitored after 24 h and 48 h after a gentle shake of the vessels. The results were used to build dose response curves and obtain EC<sub>xx</sub> values.

The results indicated that only the particles and leachates from the used CF were toxic at the concentrations used. The CF particles were more toxic than the leachates (EC<sub>50</sub> values of 0.29 and 0.38 g L<sup>-1</sup> at 48 h, respectively). Also, the particles caused a significant immobility already after 24 h of exposure compared to the leachates.

Overall, this study points at the toxicity caused by CF to freshwater ecosystems at relatively low concentrations. While recognising that the values found to cause significant toxicity would be hard to reach in large water bodies, we speculate that populations of organisms living in small ponds or puddles of water may be at risk if littering of CF continues.

#### **1.09.P-Mo096 Assessing the Effects of Car Tire Additives to a Marine Amphipod Using Behavioural Studies**

**Henry Ebele Obanya, Fay Couceiro and Alex Ford, University of Portsmouth, United Kingdom**

Behaviour in ecotoxicology is expanding as it provides a link between the biochemical and physiological effects of environmental contaminants. This has been facilitated by advancements in computational automaton and the increased prevalence of behavioural modulating compounds in the environment. The main aim of this study is to assess the effects of car tire additive compound, 2-mercapto benzothiazole (2-MBT) on the behaviours of model crustaceans, *Gammarus pulex*. A developed assay on a Zantiks unit was used to measure locomotion as a kind of behaviour associated with stress on the invertebrate. The Gammarids were exposed



to environmentally relevant concentrations (100 µg/L, 1 µg/L, 0.1 µg/L, 0.01 µg/L) of the car tire additive compound (MBT). Exposure to this compound had significant impacts on the behaviour of these Gammarids. The animals moved rapidly when light came on after 2 min, but they got slower with time. There was a significant difference found in the mean distance moved between day 7 and 14. The movement of Gammarids was affected over time with most effect found in 100 µg/L. The results obtained showed that 2-MBT affected the locomotion behaviour of the Gammarids after 7 d and 14 d even at environmentally relevant concentrations. This may then mean that the presence of these compounds in the environment may affect their behaviour in the wild as impaired locomotion can hinder their capacity to escape predation, alter reproduction, and generally affect population

#### **1.09.P-Mo097 Effects of Three Micro- and Nanoplastics Under Weathering Conditions on Messenger and Long Noncoding RNA Expression in the Inland Silverside (*Menidia beryllina*)**

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Micro- and nanoplastics (MNPs) are the result of widespread plastic pollution which has become an environmental pollutant of great concern. As plastics break down into smaller fragments the addition of weathering processes can further aid in degradation as well as alter the toxicity of the particles. However, MNP toxicity is still poorly understood and the potential mechanisms of toxicity for this class of compounds have yet to be fully explained. Additionally, how toxicity may be altered following weathering processes is even less well studied. Therefore, to better understand the potential differences in mechanisms of toxicity across MNP types, shapes, sizes, and weathering processes we have exposed the Inland Silverside (*Menidia beryllina*) to three different MNP types: tire particles (TP) and polylactic acid (PLA) at both micro- and nanosize fractions and polyester microfibers (MFs) in the microsize fraction to both weathered and unweathered forms. Micro- and nanoplastics were weathered for 72 h with exposure to UV A, B, and C while being constantly shaken in saltwater. Organisms were exposed to either weathered or unweathered particles for 21 d. Following the exposure, RNA was extracted and is currently in the process of being sequenced for both messenger and long noncoding RNA (mRNA and lncRNA, respectively). Following sequencing, bioinformatic analysis will be used to determine differentially expressed genes. mRNA will inform on the gene expression of the organisms following chronic exposure which will allow for an assessment of differentially expressed gene pathways. lncRNA play essential biological roles and are additionally important in development of diseases, the analysis of which will also provide insights into the potential epigenetic effects of MNPs. Here, we present our results which show that weathering increases or decreases toxicity may be dependent on polymer type. These data will provide a necessary comparative assessment of multiple MNP types to better understand the relative toxicity of these contaminants. Additionally, this will be the first assessment of lncRNA in the Inland Silverside, a commonly used model species in both research and regulatory testing.

#### **1.09.P-Mo098 Immunophysiological Impact of Exposure to Polyethylene Terephthalate Microparticles on Rockfish *Sebastes schlegelii***

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Microplastics (MPs) are widely detected across marine ecosystems and thus are recognized as a global problem. They are known as potential harmful factors in the marine environment, but the study of toxic pathway on their ecological impact dependent on exposure concentration is still lacking. In this study, we evaluated the toxic effects of MPs on rockfish (*Sebastes schlegelii*), which are widely distributed along the coast of South Korea. The exposure size of MP was divided into three fragment types including 0–20 µm, 45–75 µm, and >150 µm from polyethylene terephthalate (PET). After 48 h of exposure to 0.4 g/L of MPs, cytotoxicity was measured using Annexin V, propidium iodide staining, and flow cytometry. Two advanced 'omics analyses, transcriptomics and metabolomics, were also conducted to clarify toxic pathway. Differentially expressed gene derivation and validation were analyzed for transcriptome functional interpretation and component analysis. The group exposed to the smallest MP size (0–20 µm) showed the increased leukocyte apoptosis, and the phagocytic activity, measured using flow cytometry, revealed that leukocyte phagocytic activity of all exposed groups increased compared to that of the control group. Supporting the exposure response mechanism of the rockfish revealed by metabolomic analysis, correlation analysis confirmed the metabolomic changes. Our results can be used as reference data for evaluating effects caused by PET exposure and might be useful for identifying the correlation between toxic effects and physiological functional characteristics in marine fish after exposure to MPs.

#### **1.09.P-Mo099 Are *Daphnia* More Sensitive to Weathered or Pristine Microplastics Derived from Common Consumer Products?**

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A large body of research has established that microplastics (MP) are ubiquitous pollutants that can be hazardous for some species. To date, MP research has focused primarily on using commercially available pristine test materials that are often spherical and made of polystyrene, due to a lack of environmentally relevant reference materials. In the current study (REVEAL project), we investigated the toxicity of pristine and partially aged irregular-shaped MP produced from common consumer products. To obtain different materials (shape, size, polymer), two common plastic products – textile fibers and shopping bags – were selected. Fibers were made of polyester, polyamide, or polyacrylonitrile, and wool was used as natural reference fiber. For polyethylene (PE) shopping bags, we used virgin and recycled PE to investigate the influence of recycled materials on degradation and toxicity. Plastic products were cut into small pieces (<1 cm), cryomilled (6770 Freezer/Mill), and then sieved (<63 µm) prior to use in toxicity testing. Approximately half of the particles were then exposed in a UVC chamber for 20 d to simulate an accelerated

photodegradation of the particles and generate chemical changes to the surface. All MP types were characterized before (pristine) and after (weathered) photodegradation using scanning electron microscopy (SEM) images, micro-Fourier transform infrared ( $\mu$ FTIR) (carbonyl index; chemical changes), and coulter counter (shift in size distributions). Yellowing and loss of color provided visual evidence that material weathering had occurred, while an increase of carbonyl index was observed for some polymers. *Daphnia magna*, a sensitive water flea, was selected for the toxicity study. The organisms were exposed to the different MP following a standard test (OECD 211 - *D. magna* reproduction test). Reproduction, survival, growth, and O<sub>2</sub> consumption were the assessed endpoints. This study contributes to gaining a better understanding of the toxicity of weathered MP research, as well as of recycled and pristine plastics. Furthermore, the production of weathered irregular-shaped particles (film and fibers) is of great interest in MP research because of the environmental relevance compared to pristine spherical materials. More information on reference materials used can be found in the presentation “Production and analysis methods for pristine and degraded microplastic and nanoplastic reference materials,” session 3.28.

#### **1.09.P-Mo100 Embryotoxic and Neurobehavioural Effects of Nanoplastics on the Early Life Stage in Zebrafish**

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Nanoplastics (NPs) have attracted global attention due to their potential ecological and health impacts. Previous ecotoxicological studies have reported nanoplastic toxicity in various aquatic organisms but there is still limited understanding of how different nanoplastics affect embryonic development and early life stage behaviours. Here, we investigated the embryonic and neurobehavioural effects of polystyrene nanoplastics (PS-NPs) in different sizes (50 nm to 1  $\mu$ m) and with different charges (amino-modified PS-NP and carboxyl-modified PS-NP) on early-stage zebrafish. Zebrafish embryos were exposed to PS-NPs (0.1 to 10 ppm) from 2 to 120 h postfertilization. The dose-responsive toxicological assessment included mortality, hatching, deformities, locomotor activities (distance, velocity, rotation, and active time), and biochemical and transcriptomic responses. The results showed that nanoplastic exposures did not affect mortality, hatching rate, or deformities but significantly decreased locomotor activities. RNA-Seq and bioinformatical analyses predicted and characterised the key biological processes and pathways affected by different nanoplastic exposures. These findings provide new insights into the size- and surface-charge-dependent sublethal toxicity of nanoplastics and highlight the challenge of assessing the ecological risks of nanoplastics due to the great complexity of nanoplastic mixtures in the environment.

#### **1.09.P-Mo101 Effects of Plastic on the Reproductive Behaviour of the Freshwater Snail *Biomphalaria Glabrata*: An Invertebrate *in vivo* Study**

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Plastic has been an increasingly important omnipresent material with versatile properties and diverse applications since the 1950s. However, its long life and decomposition into microplastics, and its various additives cause significant environmental pollution with growing scientific and public health concerns. Risk assessment of microplastic pollution is highly demanded, however, and often includes animal testing.

In this study, we investigated the putative effects of conventional (high density polyethylene) and biodegradable plastic bags (based on corn starch) on the aquatic system, using the freshwater snail *B. glabrata*. In comparison to vertebrates, invertebrate study systems are less regulated and do not need approval by an ethical commission. Nevertheless, efforts are done to keep invertebrate research ethical. Therefore, all experiments were planned carefully and highest attention was paid to avoid any suffering. In addition, a noninvasive assay was chosen to investigate the reproduction behaviour of adult snails.

Snails were exposed to pieces of conventional and biodegradable plastic bags, as well as to leachates of the two plastic bags, which potentially contain harmful substances. The reproduction behaviour was monitored by counting the number of laid egg clutches. Our results demonstrate that the reproductive behaviour of *B. glabrata* was influenced by plastic; however the increased clutch production was possibly not affected by leached chemicals but rather by other factors such as the surface area of the plastic or the amount of plastic pieces. Although further experiments are needed to gain more information on the impact of plastic on aquatic organisms, our study demonstrates that *B. glabrata* is an appropriate *in vivo* invertebrate test system for ecotoxicological risk assessment.

#### **1.09.P-Mo102 Behavioural Toxicity of Conventional vs Biobased and Marine Recycled Polymers in European Perch and Nettle Dog Whelk**

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Microplastic is everywhere, from the beaches to the depths of the seafloor. These small plastic particles are challenging organisms and communities in a variety of different ways. By virtue of their small size, microplastics are ingested by many species, which may have adverse particle and mechanical effects. In addition, microplastics contain thousands of chemicals, which are either added during production, or which sorb to the plastics upon entering the environment. These chemicals may leach from the plastics and into the surrounding water/organisms, with the potential for additional adverse outcomes.

While much work has been done on the physiological side of toxicology, less has been done to assess the behavioural effects of exposure to plastic and its associated chemicals. Behaviour is comprised of internal stimuli and external stimuli and how these are combined to what is happening in the environment. Changes in behaviour may be detrimental to individuals and populations by interfering with chemical cues related to feeding, reproduction, or predator avoidance.

In this study, changes in behaviour will be investigated in two organisms. Firstly, a chronic exposure of juvenile European Perch (*Perca fluviatilis*) will be exposed to microplastic particles via ingestion over a six-month period. The first will be fed a mixture of conventional microplastics and biobased microplastics. Classical behavioural tests will be carried out on the perch to assess changes to behaviour. The tests will include response to a novel object, locomotion, predator avoidance, and conspecific recognition. The second study species is the Netted Dog Whelk (*Tritia reticulata*). This species will be exposed to 2% w/v chemical leachate from recycled marine plastic for two months. Predator-avoidance behaviour and response to food cues will be measured in snails exposed to either leachate or control. In addition, the snails will be assessed for the presence of imposex.

#### **1.09.P-Mo103 Toxic Effect of Polypropylene Loaded with Fluoxetine on *Daphnia Magna*: Adsorption and Desorption Mechanisms of Fluoxetine on Three Microplastic Types**

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Water quality is an increasing global environmental concern. An indicator of poor water quality is the presence of micropollutants. Pollutants, including microplastics, are commonly detected throughout freshwater and marine aquatic systems. There is an ever-developing recognition that microplastics can act as a vector for micropollutants when co-occurring and that loaded-microplastics can become integrated to food webs. This study evaluated the adsorption / desorption of the antidepressant fluoxetine with three virgin and three aged microplastic types widely reported in the freshwater environment. Microparticles of polypropylene (PP), polyamide (PA), and polyvinyl chloride (PVC) with average particle sizes of less than 33 µm were acquired commercially and artificially aged in the laboratory using a xenon arc weathering instrument (Suntest XLS+, AMETEK Inc., USA). The effect of aging microplastics on the adsorption of fluoxetine onto PP (15-100 µg/mL), PA (1-15 µg/mL), and PVC (1-15 µg/mL), followed by the desorption from the microplastics, was evaluated. Results showed similar adsorption by virgin and aged PP (83-98%), while no desorption was observed. On the other hand, fluoxetine adsorbed more to aged PA (25-68%) and PVC (38-90%) when compared to virgin PA (24-55%) and PVC (33-83%). Both virgin and aged particles of PA and PVC desorbed up to 20% of the fluoxetine from microplastics after 24 h at 25 °C. To evaluate whether microplastics loaded with fluoxetine can act as a vector for fluoxetine entering the food chain, an ecotoxicity experiment with *Daphnia magna* neonates was performed using Daphtoxkit F (Microbiotest, BE). The investigation was conducted to evaluate five treatments each containing a total of 20 neonates. After 48 h, *Daphnia magna* demonstrated ingestion of PP under all treatments where microplastic was included. However, the ingestion of microplastics did not appear to affect the mobility / survival of the organisms in the microplastic control (100 µg/mL plastic) and fluoxetine-loaded microplastic (50 µg/mL plastic). Toxic effects were observed in the treatments containing fluoxetine either in solution or loaded onto microplastics. Out of 20 daphnids, five were dead / immobilised when in a solution with only fluoxetine (1 µg/mL), and six were dead / immobilised when in a solution with fluoxetine-loaded microplastic (100 µg/mL plastic). The results indicate that microplastic can enter the food chain and act as a vector for pollutants.

#### **1.09.P-Mo104 Elucidating the Human Health Hazards of Inhaled MicroNanoPlastics in Particulate Matter Using Advanced *in vitro* Approaches**

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MicroNanoPlastics (MNPs) are formed by the biological, chemical, or mechanical degradation of plastics. Research has targeted the environmental impacts of MNPs, however the human health hazard of MNPs is largely unknown. Surgical face masks are a prevalent source of MNP exposure to humans, notably during the SARS-CoV-2 pandemic. These masks contain a high plastic content, specifically polypropylene (PP) and are known to shed MNPs. Respirable (<5 µm diameter) samples of commercial PP powder (Goonvean Fibres Ltd.), and face masks were prepared using cryomilling and cryotome slicing. Two mask samples were prepared, consisting of either all three filter layers, or just the innermost layer, and were analysed using Pyrolysis-Comprehensive-Two-Dimensional GCMS (+LECO ChromaTOF Tile) for composition, and via Zetasizer for Dynamic Light Scattering (size distribution) and Zeta Potential (surface charge). To assess MNP human impact, type-II lung epithelial cells (NCI-H441) were cultured at the air-liquid interface (ALI) and exposed to carbon black (CB) (Printex<sup>®</sup> 90), as a positive particle control, and the PP samples via aerosol (Vitrocell Cloud12) with deposited concentrations of 0.5, 1.0, and 2.0 µg/cm<sup>2</sup>. Cell cultures were then postincubated at 37°C, 5% CO<sub>2</sub> for 24 h. Alongside acellular deposition, particle, and cellular morphology (electron microscopy) assessed endpoints of cell viability (trypan blue exclusion), barrier integrity (blue dextran), (pro-)inflammatory response (IL-1β/IL-6/IL-8), oxidative stress (*SOD-1*), and genotoxicity (mononucleate micronucleus) were conducted. A decreased cell viability was observed following exposure to commercial PP and full mask samples, concomitantly with an increase in all (pro-)inflammatory mediators assessed. However, these changes were only significant at 2.0 µg/cm<sup>2</sup>, in contrast to CB, which was significantly increased between each concentration. Further, initial genotoxicity assessment suggests a minor increase to micronucleus formation for both exposure samples. Thus, data suggest that aerosolised MNPs may instigate a hazardous response *in vitro*, though the mechanisms associated with this remain under investigation. In advancement of this approach, a co-exposure of aerosolised MNPs ± (standardised) indoor and outdoor particulate matter (e.g., NIST 2584 and 1649b) upon a co-culture lung model (NCI-H441 + d-THP-1), cultured at the relevant *in vivo* ratio for the lower human lung, for 4 and 24 hr is currently ongoing.

### 1.09.P-Mo105 Detection and Quantification of Micro- and Nanoplastics in Olfactory Bulb Tissue: A Neurodegenerative Disease Association Study

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The ubiquity of micro-/nanoplastics (MNPs; 100 nm - 5mm and <100 nm, respectively) in the environment has incentivized exploration into the neurodegenerative impacts of chronic plastic exposure. There is growing concern for the distribution of MNPs in vital organs, leading to neurotoxicity, disease (e.g., cancer, renal, cardiovascular), oxidative stress, and other serious health consequences. The olfactory region of the brain is of particular interest due to its demonstrated ability to transfer neurodegenerative disease (NDD) causing agents (e.g., BMAA) to the brain via the olfactory pathway. Existing studies have observed the role of environmental toxin exposure on olfactory bulb dysfunction; however more are needed to examine specific impacts on the human brain and blood-brain barrier (BBB). This study aimed to identify and quantify concentrations of MP (BPA/TPA) monomers and plasticizers (phthalates) in postmortem olfactory bulb tissue by LC-MS/MS analysis. We hypothesized that 1) inhaled MNPs can utilize the olfactory pathway to access brain tissue, and 2) there will be significantly higher (95% CI) quantities of MPs in patients with neurodegenerative diseases compared to healthy individuals. The olfactory nerve, referred to as cranial nerve 1 (CN1), was sampled from human postmortem brain donors with documented occupational and/or residential exposure to plastic contaminants. CN1 biospecimens include donors with neurodegeneration pathologies (n = 12), as well as unaffected controls (n = 3) spanning 20 to 80 years of age. Donor selection criteria are based upon occupational and residential histories, and samples are accompanied by de-identified patient data. Tissue samples have undergone digestion, depolymerization, solid phase extraction, and elution procedures prior to LC-MS/MS analysis and quantification by isotope dilution. Identification of MNPs in the brain would be indicative of olfactory pathway absorption and could suggest a linkage between neurotoxicity and plastic exposure. The method utilized for detection of plastics in brain tissue has the potential to link MNP concentration with neurodegeneration, provided patient metadata and additional analysis to quantify neurotoxicity (e.g., single-nucleus transcriptomics, RNA sequencing). Based on the concentration of nanoplastics reported in this study, further qualitative assessment could be used to understand the neurological impact of plastic exposure.

### 1.09.P-Mo106 Combined Effects of Nanoplastics and Benzo[a]pyrene in Human Colon Adenocarcinoma Cells

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Nanoplastics (NP) and Polycyclic Aromatic Hydrocarbons (PAHs) are known to individually pose risks to environmental and human health. Interaction effects are not known, but this is a relevant issue since NP knowingly adsorb pollutants from the environment.

In this study, we propose to assess the effects of the mixture of NPs and a well-known PAH, the benzo[a]pyrene in HT29 cells (human colon adenocarcinoma cell line). First, cellular viability (MTT assay) was evaluated exposing HT29 cells to five different concentrations of polystyrene NP (25, 50, 100, 200, and 500 g.ml<sup>-1</sup>) with 25, 50, and 100 nm of diameter and B[a]P (1, 5, 25, 50, and 100 µM) individually, for 24, 48, and 72 h. Secondly, several biomarkers related with the detoxification mechanisms of B[a]P, like 7-ethoxyresorufin O-deethylase (EROD), glutathione-S-transferase (GST) activities and glutathione levels (reduced [GSH] and oxidized [GSSG] forms) were afterwards analysed in cellular lysates exposed to 500 g.ml<sup>-1</sup> of NP and two B[a]P concentrations (5 and 50 µM) individually and in mixture.

The results revealed that larger NP particles (100 nm) yielded higher cytotoxicity than smaller plastics. The cytotoxicity increased with time of exposure to both contaminants. Results showed higher EROD activity in cells exposed to NP (with and without B[a]P). Contrary to expected, cells exposed to NP and B[a]P mixtures showed lower EROD activity comparing to NP individual contamination. This can occur due to surface interactions decreasing B[a]P accessibility. On the other hand, GST activity increased with the increase of B[a]P concentration and in exposure containing the mixture of both contaminants (B[a]P and NP), pointing to increased conjugation of metabolites.

The results highlighted the complex nature of the interactions between NP and B[a]P.

### 1.09.P-Mo107 Study of the Bioavailable Fraction of Metals Derived from Polylactic Acid (PLA) Microplastics

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The production of degradable polymers is rising considerably in recent years seeking to replace nondegradable polymers and to solve many of the widely known environmental problems arising from their use.

Poly(lactic acid) (PLA) is considered one of the most commercially promising bioplastics, due to its wide availability, tunability, and lower production cost compared to other biodegradable polymers. It is derived from renewable sources (sugar and starch) from which lactides are obtained, followed by a conventional polymerization process. In this step, a high amount of metallic catalyst is required, such as lead, tin, zinc, or aluminum, which can remain as impurities in the final product.

On the other hand, although PLA is compostable, it can remain in the marine environment for up to 1000 years, generating a great amount of microplastics that can enter the food chain.

In this framework, it would be necessary to assess the potential risk derived from the metals presented in bioplastics such as PLA. Then, metal contents in PLA samples were tested in the present study, observing the elevated Sn concentration (ca. 25 µg/g). Moreover, bioavailable metallic fraction was assessed by using an *in vitro* human digestion procedure, following the *in vitro* unified BARGE Method. This method simulates the human digestive procedure using synthetic digestive fluids (saliva, gastric, duodenal, and bile) and, in this work, a dialyzing membrane step was included to estimate the possible metallic diffusion transport into the simulated blood fluid. This allows discerning between the bioaccessible (BARGE Method) and bioavailable fractions.

#### **1.09.PC Micro (Nano)Plastics: Occurrence, Fate, Uptake, and Mechanistic Approaches to Understand Their Risk for the Environment and Human Health**

##### **1.09.P-Mo052 Modelling Environmental Microplastic Fate and Exposure**

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Exposure assessment is the basis of risk assessment for microplastic particles (MP). Since small microplastic and nanoplastic particles are almost impossible to detect, and their concentrations can only be estimated by interpolating in time and space, retrospective risk assessments must rely on modelling. This requires a holistic modelling approach, linking sources, transport, fate, and exposure of MP. In this research, we will develop and validate a range of microplastic transport and fate models, encompassing different spatial and temporal scales. The first step will be to develop a harmonized microplastic emission model, followed by the development of a 'unit world' multimedia model. Microplastic fluxes generated by this multimedia model will serve as input for a detailed spatiotemporal river transport model. While previous models were usually parameterized to simulate only the behaviour of monodisperse spherical microplastic particles, we will apply new approaches using probability density functions (PDFs). Using these PDFs we will be able to capture the full multidimensionality and diversity of environmentally relevant MP as they exist in nature. Modelled MP-PDFs can then be tuned to threshold effect concentrations to characterize ecological risks in environmental systems. The toolset presented enables us to prospectively assess risks for decades to come.

#### **1.09.V Micro (Nano)Plastics: Occurrence, Fate, Uptake, and Mechanistic Approaches to Understand Their Risk for the Environment and Human Health**

##### **1.09.V-01 Accumulation in Soft Body, Histological Alteration, and Cytotoxicity Caused by Exposures to Two types of Microplastics in the Mediterranean Mussel (*Mytilus galloprovincialis*)**

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Plastics enter the ocean through various routes and are decomposed through physicochemical reactions such as waves, ultraviolet, temperature, and pH. Due to this reaction, various sizes and shapes of microplastics (MPs) and nanoplastics (NPs) are formed, which easily accumulate in the organism body and cause toxic effects. In the study, we investigated the effects of exposures to two types (spherical and fiber) of MPs on Mediterranean mussel (*Mytilus galloprovincialis*). To do this, we observed biological and physiological responses including survival rate, number of MPs accumulation in the soft body, and histological structures on *M. galloprovincialis* exposed to each MP type. The survival rate decreased on *M. galloprovincialis* following exposures to two types of MPs as a dose-time dependent manner. It is a direct toxic effect of exposed MPs. Especially, a significant decrease of the survival rate was observed on *M. galloprovincialis* exposed to the fiber type, suggesting that it has higher toxicity than the spherical type. As a result of MPs accumulation in the soft body, accumulation of spherical MP decreased until the 7 days, but a sharp increase of the MP particles observed on *M. galloprovincialis* body at 21 days. However, body accumulation of fiber type tended to increase throughout the exposure periods. The exposure to two types of MPs induced alterations of histological structures including increased width of gill filament in the gill tissue of *M. galloprovincialis*. We also observed the cytotoxic signals in gill tissues of all exposed groups as a time-dependent trend using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). These results suggest that exposures through two types of MPs induced different accumulation sensitivities on *M. galloprovincialis* body and were directly related to survival changes. Moreover, observation of histological alterations in gill tissues by inductions of cytotoxicity is a good tool to assessing MPs pollutions in aquatic environment.

##### **1.09.V-02 Leaching of Microplastic Constituents in Aqueous Medium; Aquatic Toxicity on Marine and Freshwater Organisms**

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Sources of microplastic (MP) in the environment are various including inefficient wastewater treatment plants, and stormwater runoff. Also, everyday activities that produce municipal plastic waste due to unregulated waste management end up in the aquatic environment which with time can be degraded and fragmented into MP particles. Due to its shape and size, MP can easily enter the food chain and cause negative effects on the organisms. Also, it is important to notice that in atmospheric conditions waste

polymer material as well as MP can release additives that are mostly residue from the synthesis or even can be produced in the process of recycling.

The aim of this study was to investigate the qualitative content and aquatic toxicity of samples during the leaching process. For that purpose pristine and photooxidative (UV) aged polyethylene terephthalate (PET) bottles were exposed to the enhanced atmospheric conditions. In leaching tests, using pristine and aged MP, the concentration of bisphenol A (BPA) in the aqueous phase was monitored using High-Performance Liquid Chromatography Analysis. Heavy metal concentration was monitored using Inductively Coupled Plasma Mass Spectrometry. Acute toxicity bioassays were performed with the freshwater microalgae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) and the crustacean *Daphnia magna* as well as marine bioluminescent bacteria *Vibrio fischeri*, according to standard procedures (ISO).

Obtained results indicate that the fragmentation of PET bottles is enhanced for pristine PET bottles in terms of a higher percentage of smaller particles. Leachates that are released from both pristine and aged PET samples were BPA and antimony. The release of antimony was much lower from the UV-aged sample. In general, presence of leached constituents enhances overall toxicity effects in comparison with the samples that consisted of only MP particles.

These results will serve as preliminary study for the further investigation of microplastic behaviour in the aquatic environment and the goal is to step forward to the more complex environmentally relevant samples which are considered as a mixture of microplastics and other pollutants.

#### **1.09.V-03 Influence of Photooxidative Aging on the Aquatic Toxicity of Microplastic Tested on Freshwater Organisms**

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Everyday life conveniences and activities such as washing clothing made of synthetic fibers, tire wear, and plastic packaging break down in the environment may serve as an abundant source of microplastic (MP). There is plenty of evidence that microplastic is a problem due to its shape, size, and concentration and the fact that it can easily enter the food chain which causes negative impacts on human health and the environment. An important aspect of this problem is also the fact that microplastic and other contaminants of emerging concern (CEC) present in water can interact, in terms of the adsorption of CEC onto the MP surface. The adverse effect of MP in water is therefore influenced by factors affecting adsorption capacity: particle size, specific surface area, hydrophobicity, and polarity of MP as well as CEC.

The aim of this study was to investigate the aquatic toxicity of pristine and photooxidative (UV) aged microplastics and pesticide atrazine samples after the sorption. For that purpose, we investigated the impact of UV aging on polyethylene terephthalate (PET) bottles and correlated it with surface properties. Polyethylene terephthalate was inspected for morphology changes using Scanning Electron Microscopy and Fourier Transformation Infrared Spectroscopy. The specific surface was investigated by using Brunauer-Emmet-Teller Analyzer to determine the adsorption capacity of MP. The fragmentation of PET bottles into microplastic particles has also been investigated. In adsorption tests, using pristine and aged PET MP, the concentration of atrazine in the aqueous phase was monitored using High-Performance Liquid Chromatography Analysis. Aquatic toxicity samples were assessed by exposing freshwater organisms to the aqueous dispersion of pristine and aged MP, without and with adsorbed pollutants. Acute toxicity bioassays were performed with the microalgae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) and the crustacean *Daphnia magna* according to standard procedures (ISO).

Obtained results indicated that UV aging resulted in changes in PET bottle morphology and structure. Carboxyl groups are confirmation of surface degradation. The adsorption capacity of MP increases with a higher aging degree. Adsorption capacity is decreasing with an aging degree. Toxicity is increasing with the complexity of samples but decreases with the aging degree. Mixture (MP and CEC) confirmed higher toxicity in comparison to the individual components.

#### **1.10 Nanoparticle Biological Interactions and Their Responses**

##### **1.10.P-We001 Intracellular Measurement of Dissolved Metals and Localization of Metal Nanoparticles using X-ray Spectroscopy Techniques**

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Intracellular dissolution of metal nanoparticles (NPs) and speciation of dissolved metal species relates to cellular toxicity and bioreactivity. While it is possible to predict the chemical transformations of metals and metal NPs in the extracellular environment, intracellular chemical transformations are more challenging to predict. X-ray absorption spectroscopy (XAS) is optimally suited for the nondestructive determination of the speciation of metals in situ. Moreover, imaging of intracellular metal and metal nanoparticle can be achieved by X-ray fluorescence microscopy (XRF). The intracellular fate of NPs might have important toxicological implications, but it remains poorly understood. In this study we used an intestinal cell line from rainbow trout (*Oncorhynchus mykiss*), the RTgutGC. We used the RTgutGC cells and XRF to determine the intracellular localization and concentration of essential trace metals in cells exposed to silver and titanium dioxide NPs. Cell monolayers were exposed to nontoxic and toxic concentrations of silver nitrate and silver and titanium dioxide NPs. After exposure, cells were washed and

then fixed chemically or cryogenically. To determine the intracellular NPs' localization, a subset of samples was measured using 3D tomography. In a parallel set of samples metal intracellular concentration was measured by ICP-MS. The comparison of metal quantification by XRF and ICP-MS in RTgutGC cells showed a remarkably similar concentration of trace metals. X-ray fluorescence imaging of cells exposed to silver nitrate showed a reduced nuclear size confirming previous imaging data. Cells exposed to silver and titanium dioxide NPs accumulated nanoaggregates intracellularly. Remarkably, titanium dioxide NP aggregates showed a significant increase in the trace metals iron and zinc suggesting that these metals could sorb onto the NP surface. The 3D tomography data demonstrated that both silver and titanium NPs can translocate to the cell nucleus. Although technically challenging, XRF imaging is a very powerful technique and can be used to accurately map intracellular metal concentrations. Although titanium dioxide NPs are not acutely toxic their sorption properties might result in disruption of essential trace metal metabolism. The nuclear translocation of silver and titanium dioxide NPs is alarming but could also be exploited for certain applications.

#### **1.10.P-We002 Size and Shape-Dependent Interactions of Lipid-Coated Silver Nanoparticles: An Improved Mechanistic Understanding Through Model Cell Membranes and *in vivo* Toxicity**

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The wide applicability and versatility of silver nanoparticles (AgNPs) has led to an increased demand for optimized AgNPs with tuned physical, optical, and chemical properties for various industrial and biomedical applications. Specifically, biocompatible and stable AgNPs with minimal impact on human health and the environment are of significant interest. It is well-known that the physical, optical, and catalytic properties of AgNPs are strongly influenced by their size, shape, and surface properties. While *in vitro* and *in vivo* studies of AgNP uptake have provided a wealth of knowledge regarding the mechanism and efficiency of AgNP uptake, distribution, and toxicity, many of these studies cannot disentangle the contribution of silver ions to these processes or effects.

Our approach was to systematically study a series of engineered AgNPs that are shielded from dissolution, detailing their interactions with i) biomimetic lipid monolayers and their ii) uptake and effects *in vivo*. Well-studied biomimetic lipid monolayers have been employed to assess the interactions of biomolecules or NPs with a lipid monolayer. Sum Frequency Generation (SFG) vibrational spectroscopy can detect the adsorption and orientation of biomolecules at submicromolar concentrations and can directly identify interactions between NPs and model cell membranes with molecular resolution. For these studies, we synthesized a size and shape series of AgNPs coated with robust and tightly packed lipid bilayer composed of sodium oleate (SOA), hydrogenated phosphatidylcholine (HPC), and hexanethiol (HT).

Combining *in vivo* toxicity studies with SFG spectroscopy and model membranes we were able to elucidate our gap in knowledge in understanding of how NP shape and size influence interactions without the confounding effect of ionic Ag. We observed differences in toxicity related to both the size and shape of AgNPs without the confounding factor of dissolved Ag ions being present. Zebrafish exhibited hyperactive spontaneous movement from the AgNP exposures and a unique caudal fin malformation was observed in both the AgNS and the AgNPL. This work demonstrates the utility of *in vitro* evaluations with biomimetic models combined with *in vivo* testing to move toward mechanistic understanding of biomaterial interaction. This study further supports the need for moving towards mechanistic evaluations of nanomaterial technology to inform rational design, hazard, and application.

#### **1.10.P-We003 Assessment of Multiple Stressors: Combined Effects from Exposure to Silver Nanoparticles or Zinc-Oxide Nanoparticles with the Pathogen *Klebsiella Pneumoniae* in *Caenorhabditis Elegans***

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Nanoparticles (NP), such as AgNP and ZnONP, are rapidly being incorporated into agricultural settings as precision herbicides and pesticides. To assess the potential toxicity of these NP, model organisms are exposed to NP as a single stressor, but in nature, organisms are affected by multiple sources of stress, including infections, which might exacerbate or mitigate negative effects of NP exposure. To expand our understanding of the environmental consequences of released NP, this project examined the combined effects of AgNP or ZnONP on *Caenorhabditis elegans* infected with a common pathogen, *Klebsiella pneumoniae*. To determine effects of single stressors on reproduction, nematodes were exposed to AgNP or ZnONP while being fed nonpathogenic bacteria. Nematodes were also fed *K. pneumoniae* without the presence of NP. All exposure treatments decreased reproduction in nematodes. To assess effects of combined stressors, *C. elegans* were exposed to EC<sub>30</sub> concentrations of AgNP or ZnONP along with the pathogen. Exposure to both AgNP and *K. pneumoniae* resulted in reproduction that was not significantly different compared to single stressors exposures. However, combined exposure to Zn treatments and *K. pneumoniae* resulted in reproduction that was not significantly different from controls. Further experiments aimed to determine mechanisms of the antagonistic effects in Zn treatments. Mitigation of the pathogen effects by ionic or particulate forms of Zn is partially explained by the estimates of the colony forming units (CFU) of *K. pneumoniae* within treated nematodes. The nematodes exposed to ZnONP had significantly lower CFU compared to nematodes only exposed to *K. pneumoniae*. Pathogen biofilm formation after Zn exposure was also assessed. Results showed that *K. pneumoniae* produces biofilm, even in the presence of Zn (ion controls or ZnONP); however, Zn exposure significantly lowered biofilm production. Taken together, our results suggest Zn exposure decreases *K. pneumoniae* ability to form biofilms in host intestines, yet mechanisms of reduction of host Zn toxicity by pathogen

exposure remain unknown. To elucidate molecular pathways that are involved in the observed antagonistic effects, transcriptomic data from nematodes exposed to Zn and *K. pneumoniae* is currently being analyzed.

#### **1.10.T-01 Bionano Interactions: A Key to Mechanistic Understanding of Nanoparticle Toxicity**

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The new paradigm in the assessment of toxicity of nanomaterials relies on a mechanistic understanding of the organism's response to an exposure to foreign materials from the initial, molecular level interactions to signaling and regulatory cascades. Such detailed information is often missing as only downstream events of adverse outcome pathways (AOP) are registered. Physics-based molecular modelling could provide such information, which can, in its turn, serve to generate the data for a development of quantitative structure-activity relationships between the nanomaterial properties and the key events of the AOPs and prediction of toxicity for new materials. Recently, a significant progress has been made in development of multiscale physics-based models of bionano interactions designed to fill the gaps in description of the toxicity mechanisms. Here, we present a methodology to quantify the essential interactions at the bionano interface using multiscale modelling, which can be used in combination with the AOP analysis to build mechanism-based predictive schemes for toxicity assessments. We introduce a set of new, advanced descriptors of the nanomaterials, which refer to their ability to bind biomolecules and trigger the pathways via the molecular initiating events. We also present a suite of open-source software tools to evaluate the interactions of biomolecules with arbitrary nanomaterials and predict the binding affinity and content of biomolecular corona for those materials. We demonstrate the predictive power of the *in silico* methods and novel descriptors by modelling various *in vitro* and *in vivo* biological endpoints.

#### **1.10.T-02 Mechanistic Insight for Microbiota-Inclusive Nanotoxicology: Metabolite Corona and TLR2 Signaling**

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Recent progress in the nanosafety field revealed the importance of interactions between hosts and their associated microbiota to nanotoxicity. Mechanistically, microbiota-dependent nanomaterial toxicity has mainly been related to nanoparticle-induced dysbiosis, that is, to changes in the composition of host-associated microbiota that have detrimental consequences to host physiology (e.g., causing colitis or obesity). Conversely, nanoparticles have also been found to cure dysbiosis in plants and animals, thereby improving crop yield, preventing diarrhea in cattle, and inhibiting dental caries formation. In this presentation, I will introduce two other mechanisms that govern microbiota-dependent nanomaterial toxicity. The first of these mechanisms, is the adsorption of microbial metabolites onto the surface of nanomaterials, potentially affecting the identity and colloidal stability of nanoparticles as influenced by the biocorona. By constructing quantitative structure-activity relationship models, we predicted the adsorption affinities for 60 microbial metabolites to 13 metal and 6 carbon nanomaterials. Correlations of these predictions with surface interaction descriptions revealed key interaction types that drive the adsorption of microbial metabolites to nanomaterials. Using molecular dynamics simulations, we obtained excellent support for these interaction types, and moreover showed how flexible metabolites can gain stability on the nanomaterial surface via conformational changes. The second microbiota-dependent toxicity mechanism that we investigated, is the recognition of microbiota by Toll-like receptors (TLRs), potentially modulating the immune response of microbially-colonized hosts to immunotoxic nanomaterials. We studied this by comparing the sensitivity of wildtype zebrafish larvae, to that of mutants for TLR2, and TLR2-adaptor proteins (TIRAP and MyD88), to immunotoxic silver nanoparticles (nAg). This revealed that, exclusively under microbially colonized conditions, mutants for TLR2, and for one of its adaptor proteins (TIRAP), were more sensitive to nAg than their wildtype siblings. This indicates that TIRAP-dependent TLR2 signaling can protect hosts against immunotoxic nanoparticles. Based on common extrapolation strategies for nanotoxicology, I will present how this insight into these two microbiota-dependent toxicity mechanisms can contribute to more accurate predictions of nanomaterial safety for human and environmental health.

#### **1.10.T-04 Investigating the Impact of Environmental Transformation of Nanomaterials on Their Fate of Nanomaterials Using Isotopic Enrichment and a pilot Wastewater Treatment Plant**

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An increasing number of consumer products, such as cosmetics, textiles, and nanomedicines contain engineered nanomaterials (ENMs). Engineered nanomaterials can end up in wastewater from where they are removed quite efficiently to the sludge by wastewater treatment plants (WWTPs). Most ENMs are chemically and physically transformed in wastewater, they can still potentially have effects on aquatic organisms and their dissolvable metals still appear to be bioavailable. However, only a few studies have investigated transformed ENMs. To date, most existing studies on ENMs bioavailability or accumulation were performed with pristine ENMs and/or the studies were conducted with artificial wastewater to simulate the transformation processes in WWTP. In addition, most investigations used unreasonably high ENM concentrations. The aim of this study is to



investigate the fate of ENMs in WWTPs and the impact of the environmental transformation processes that occur in WWTPs at realistic ENM concentrations through the use of stable isotope labelling technique.

To this end, isotopically enriched ENMs ( $^{109}\text{Ag}$  and  $^{68}\text{ZnO}$ ) were synthesized and used. This allows ENM tracing and detection at low concentrations in complex matrices, even against the high background levels of elements present in WWTP matrices. To investigate the fate of the ENMs within the WWTP system, a pilot WWTP that operated with municipal wastewater was used.

The produced WWTP matrices (effluent and digested sludge) containing the enriched transformed ENMs were employed in exposure tests with the benthic amphipod *Hyaella azteca*, which was previously used in various studies on the bioaccumulation of ENMs, also in combination with WWTP matrices. Data derived from body burden measurements were then used to compare the availability of the respective metals from ENMs that passed through the WWTP.  $^{109}\text{Ag}$  and  $^{68}\text{ZnO}$  from ENMs that passed through the WWTP or were exposed with WWTP matrices (e.g., spiked to control effluent) showed a strongly reduced availability compared to their pristine counterparts. In contrast, the accumulation for non-enriched Au ENMs (also studied) was higher for ENMs that passed the WWTP. The experiments hence show that lab scale pilot WWTPs can be used to investigate the fate of ENMs in WWTPs and that the use of isotopically enriched ENMs allows monitoring of ENMs even at low and environmentally relevant concentrations.

## 1.10.P Nanoparticle Biological Interactions and Their Responses

### 1.10.P-We004 Potential Issues Specific to *in vitro* Toxicity Tests of Cellulose Nanofibers

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Cellulose nanofibers (also called cellulose nanofibrils or nanofibrillated cellulose; CNFs) are a natural material consisting of a soft and long structure originating from wood or plants. These materials have low weight, high tensile strength, and a low thermal expansion coefficient. Various applications have been proposed to take advantage of these unique features. However, the introduction of new materials into the market requires thorough safety studies to be conducted. Recently, toxicity testing using cultured cells has attracted attention as a safety assessment that does not rely on experimental animals. Recent literature regarding CNF cytotoxicity tests was reviewed and the current toxicity assessment state surveyed. In the literature, a variety of cell lines and CNF exposure concentrations were found and selected. Furthermore, the cytotoxicity test results differed and were not necessarily consistent. Numerous examined reports had not evaluated endotoxin/microbial contamination or CNF interaction with the culture medium used in the tests. The following potential specific issues involved in CNF *in vitro* tests were discussed: (1) endotoxin contamination, (2) microbial contamination, (3) culture medium component adsorption to CNFs, and (4) changes in aggregation/agglomeration and CNF dispersion states resulting from culture medium components. In this presentation, the available measurement methods and solutions for these issues will be discussed. Addressing these points will lead to a better understanding of cellular effects of CNFs and safer CNF development.

### 1.10.P-We005 The Importance of a Comprehensive Toxicity Assessment of Nanopesticides in the Environment: Comparing Toxic Effects of Tebuconazole in Different Formulations on the Nematode *C. elegans*

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Nanopesticides (NPs) are considered less environmentally harmful formulations compared to existing conventional plant production products, able to control better the exposure of pests, reduce the total amount of pesticides consumed, and increase the environmental balance in agriculture. However, like any other new materials, these new nanoformulations of pesticides also need to be evaluated very carefully for their possible environmental risks prior to reaching the market. Nematodes are the most abundant and species-rich soil metazoans and are key drivers of soil functioning. The nematode *C. elegans* is used as a test organism with sublethal endpoints to assess chemical toxicity in water and soil. This invertebrate is used as a model due to its advantages which fill the gap between *in vitro* and *in vivo* tests, in addition to the simplicity, accuracy, repeatability, and low cost of the experiments. We tested the toxicity of two different NPs with polymer and lipid nanocarriers encapsulating fungicide tebuconazole (TBZ) as the active ingredient and a commercial TBZ formulation to *C. elegans* in different TBZ concentrations in the aquatic matrix. Additionally, the nanocarriers without TBZ were also used to assess their toxicity on *C. elegans*. We designed tests based on worst case scenario, so the nematodes were exposed to maximum released TBZ into the media after 48 h. Both tested NPs showed similar toxicity. However, experiments with the pure carriers revealed that the higher toxicity of NLC-TBZ was mainly due to stronger effects of NLC- compared to PCL-carriers, suggesting mixture toxicity of nanoparticles and active ingredient. Overall, these *in vivo* tests highlight that comprehensive toxicity testing of NPs is required to allow a reliable risk assessment.

### 1.10.P-We006 Size- and Concentration-Dependent Disturbance in Algal Metabolism Induced by nTiO<sub>2</sub> Exposure

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The present work aims to fill an important knowledge gap regarding the metabolic perturbations induced by engineered nanomaterials on phytoplankton species. More specifically the goal is to decipher the metabolic perturbations in green alga *Chlamydomonas reinhardtii* induced by exposure to nTiO<sub>2</sub> materials and to explore if size- and concentration-dependences of the observed effects exists. To this end, green alga *C. reinhardtii*, grown in 4xTAP medium was exposed to increasing concentrations

(2 to 200 mg L<sup>-1</sup>) of nTiO<sub>2</sub> with primary sizes of 5, 15, and 20 nm for 72 h. The effects of nTiO<sub>2</sub> on the algal growth, oxidative stress, membrane permeability, and photosynthetic activity were determined. In parallel, the stability of nTiO<sub>2</sub> suspensions in exposure media was followed by measuring hydrodynamic size distributions and Z-potential, as well as the sedimentation rates of nTiO<sub>2</sub>. The metabolic perturbations in *C. reinhardtii* exposed to nTiO<sub>2</sub> of different sizes and concentrations were determined by liquid chromatography mass spectrometry. Transcriptomic responses of *C. reinhardtii* exposed to nTiO<sub>2</sub> were assessed using NanoString nCounter Technologies. Experiments with unexposed controls were also performed. The results revealed that despite the important aggregation and sedimentation, the exposure to increasing concentrations of nTiO<sub>2</sub> with primary sizes of 5, 15, or 20 nm altered significantly the abundance of metabolites involved in various pathways corresponding to amino acid, nucleotides, fatty acids, TCA, and antioxidant metabolism. Most of the responsive metabolites were common for the all treatments; however the intensity of the response and the existence or not of concentration- and nanoparticle primary size-dependences differed among the treatments. The metabolomics results correlate well with the transcriptomics and physiological results and confirmed that oxidative stress is a major toxicity mechanism for nTiO<sub>2</sub> exposure. The implications of the obtained results for the assessment of the toxicity and tolerance responses in phytoplankton and for enabling a discovery of sensitive markers for early warning are discussed.

#### 1.10.P-We007 Sublethal Effects of Polyesterene Nanoplastics on Freshwater Organisms

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The global accumulation of micro and nanoplastics is a growing concern for the environmental and human health. While the toxicity of macro and micro-sized plastics is relatively well studied, toxicity of nanoplastics is largely uncharacterized. This study investigated the possible size-related effects of 100 and 200 nm polystyrene nanoparticle (PS-NPs) standards using cyanobacteria *Microcystis aeruginosa* and *Danio rerio* zebrafish as model organisms evaluating, not only mortality or growth inhibition, but also structural modifications, PS-NPs interactions with the organisms (SEM), and impact on toxin biosynthesis or availability (microcystin quantification by ELISA). To make possible the comparison and reusability of the data reported, standard testing guidelines TG201 and TG236 were employed for ecotoxicity assays and the stability of the PS-NPs dispersion in the test media was always characterized (DLS, z-potential and NTA).

No apparent acute toxicity was registered between the two PS-NPs in the model organisms; however results demonstrate that both PS-NPs have an influence on *M. aeruginosa* toxin microcystin availability in water, being different for both sizes. Additionally, an unprecedented structural modification on zebrafish embryonic development in response to both PS-NPs at the highest concentration tested (100 mg/L) was confirmed; the appearance of highly elongated neuromasts.

#### 1.10.P-We008 Exposure Scenarios During Aging of Titanium Dioxide Nanomaterials Affect Dimethoate Toxicity in *Daphnia*

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Titanium dioxide nanomaterials (nano-TiO<sub>2</sub>) are frequently used in consumer products (e.g., cosmetics, cleaning agents, filters, food, etc.) and are consequently released into freshwater systems via wastewater treatment plants. Furthermore, insecticides may enter freshwater ecosystems via runoff from agricultural fields. During their aquatic life cycle, nano-TiO<sub>2</sub> may interact with co-existing insecticides, simultaneously undergoing aging processes (over time) and may alter the toxicity of insecticides towards nontarget organisms. The effect sizes of these interactions may also be regulated by environmental factors, including natural organic matter (NOM) and ultraviolet (UV) radiation. The present study aimed at assessing the impact of NOM, UV radiation, and nano-TiO<sub>2</sub> over time (aging) on the toxicity of the insecticide dimethoate using a factorial test design. The test design involved two levels of NOM (0 versus 8 mg/L TOC), two levels of UV radiation (0 (dark) versus 18 ± 1 W/m<sup>2</sup> (8 h UV: 16 h dark cycle)), three nano-TiO<sub>2</sub> levels (0.0, 0.6 and 3.0 mg/L), four aging durations (0, 1, 3, and 6 days) and seven nominal dimethoate concentrations (ranging from 0 – 64 mg/L). Eventually, the ecotoxicity of the aged medium was assessed using *Daphnia magna* as a model organism. The results showed that the presence of nano-TiO<sub>2</sub> (irrespective of its concentration, NOM, and UV radiation levels) significantly mitigates dimethoate toxicity by a factor of 2 relative to an unaged dimethoate solution in absence of nano-TiO<sub>2</sub>. Upon aging in the darkness, these effect sizes did not exceed a factor of 3 after 6 days of aging. Contrary, after 6 days of aging in presence of UV, NOM, and nano-TiO<sub>2</sub>, dimethoate toxicity was reduced up to 5-fold. While other environmental factors may additionally influence the interaction of nano-TiO<sub>2</sub> and dimethoate, it is evident from this study that the presence of both NOM and UV radiation along with higher aging duration assist this nanomaterial in mitigating dimethoate-induced toxicity.

#### 1.10.P-We009 The Use of an *ex vivo* Gut Sac Technique to Determine the Uptake of Nanogold in the Gastrointestinal Tract of *Clarias gariepinus* (African Sharptooth Catfish)

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Nanotechnology is a rapidly growing science with increasing application in the field of nanomedicine. Nanogold is commonly used engineered nanomaterial (ENM) in drug delivery and has received special attention due to its unique properties. There is

however a risk of ecological exposure to these materials as a result of unintended releases into the environment. It therefore becomes essential to evaluate the behaviour, biodistribution, and bioaccumulation of these ENMs. The gastrointestinal tract is an important exposure pathway for ENMs in fish and this study made use of the *ex vivo* gut sac methodology to evaluate the uptake of nanogold and its metal equivalent in the African sharptooth catfish (*Clarias gariepinus*). Gut sacs were prepared and exposed to 1 mg/L nanogold and ionic gold for a period of 96 h. The gold concentrations in different sections of the GIT (mucosa and serosa of the stomach, anterior- mid and posterior intestine) were determined using ICP-MS. Significantly higher gold concentrations (compared to the control) were present in all regions of the GIT following exposure to ionic gold indicating uptake of gold. With the exception of the mid- and anterior intestine mucosa, no gold was detected following exposure to nanogold. The presence of gold particles was verified using CytoViva darkfield imaging. It is likely due to the particles becoming entrapped in the mucosa rather than actual uptake. In contrast, ionic gold accumulated in the muscularis suggesting the transepithelial transport. The gut sac method successfully demonstrated that there was not uptake of nanogold through the GIT of the African sharptooth catfish.

#### 1.10.P-We010 Understanding the Impact of Nanoparticle Charge on Algae-Nano Interactions Using Functionalized Carbon Dots and Morphological Data

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Nanoparticle surface charge is a critical factor influencing the interactions of nanomaterials and biological systems. Understanding the role charge plays in these interactions is key to understanding the ecological risks of nanoparticle exposure and informing sustainable nanoparticle design. In this study, the impact of nanoparticle surface charge on nano-algae interactions was investigated, using carbon dots (CDs) functionalized with polymers to have positive, negative, or neutral surface charge and the freshwater algae *Raphidocelis subcapitata* as models. Morphological impacts on green algae were investigated utilizing high-throughput fluorescence microscopy. Results indicate that positively charged CDs are most toxic to green algae, but that CDs with negative or neutral charge still induce sublethal impacts on algae. Specifically, morphological data suggests that exposure to negatively charged CDs leads to increased neutral lipid droplet formation. These lipid droplets are produced primarily when algal cells undergo stress. However, the mechanism by which functionalized CDs assert toxicity is still unclear. Further linking of morphological traits to outcomes such as toxicity can inform sustainable nanoparticle design.

#### 1.10.P-We011 Natural Compounds, Metal Complexes, Nanoencapsulation, Release into The Environment

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The world supply of agricultural products and the control of disease transmission by insects are highly significant. Pest ants are one of the main problems for Brazilian agriculture, causing enormous damage to agribusiness. *Aedes aegypti* is responsible for transmitting dengue, yellow fever, chikungunya and zika virus in the world. New chemical and biological techniques are being developed specially to reduce costs, the amount of insecticides applied and release into the environment. Studies to control ants and *Aedes aegypti* without contaminating the environment have been carried out by the natural products group and its collaborators with significant results, such as tests to determine the toxicity in *Brachydanio rerio* of the insecticides obtained, or nanoencapsulations to stabilize the active compounds and reduce the amount used, and obtaining complexes of natural products with metals to increase the insecticidal action and reduce the amount applied. In these studies, compounds from different classes of plants and co-cultures were obtained, such as flavonoids, coumarins, terpenes, chalcones, anthraquinones and chromone were the most active as insecticides, fungicides and bactericides, and many of which were complexed with metals.

#### 1.10.P-We012 Ecotoxicity Assessment of Dust from Inorganic Aerogel Mats

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European Union initiatives are developing and integrating 'green' practices to promote a toxic-free environment from the early stages of product development. Given the novelty of some materials, challenges are being faced in the assessment of advanced materials that are safer for us as well as for the environment from their design until their end-of-life stages, and still achieve the required performance. Examples of such materials are inorganic aerogels, who have emerged as key components of innovative and high-performance insulating mats, starting to replace conventional alternatives in the construction field.

One of their main advantages related to sustainability is their low thermal conductivity (less than 0.02 W/mK), compared to 0.025 - 0.040 W/mK seen in conventional benchmarks. This enables lower energy consumption and cost savings from heating and cooling of indoor air, leading to more efficient and sustainable cities. Yet very little is known about the potential exposure scenario for the environment at the end of life, during demolition and disposal. This can lead to potential risks to surface water organisms, if any of the components or their degradation products should be bioaccumulating, or persistent, or toxic or mobile.

To our knowledge, no previous ecotoxicity studies have been conducted for dust released from aerogel materials. Hence, here we investigate the potential environmental effect of three commercial silica-based and fibre-supported aerogel mats. The dust released aerogel particles were obtained from studies conducted by the H2020 HARMLESS project (GA 953183), with sizes ranging from submicron to micron particles. The environmental exposure was assessed through ecotoxicity testing of the aerogel particles in aquatic media. *Daphnia magna* 48 h immobilisation test and freshwater alga 72 h growth inhibition test were

conducted following OECD guidelines. Our findings suggest that inorganic aerogel particles show non-significant toxic response on *D. magna* and *Pseudokirchneriella subcapitata*, indicating no adverse effects on aquatic environment.

#### 1.10.P-We013 Do Coatings on Copper Oxide Nanomaterials Impact Their Fate and Effects in Soil?

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Metal oxide nanomaterials can be found in an increasing variety of applications and are frequently engineered with coatings to improve their stability, dispersability and resistance to weathering or, to provide functionalized surfaces for specific applications. As the production and use of these engineered nanomaterials increase, so does concern over their fate in the environment. There is mounting evidence that the effects of metallic nanomaterials on soil dwelling organisms can be attributed to the release of metal ions. However, it is unclear if this relationship is maintained when the nanomaterials are coated. This research examined the fate and effects of three differently coated (polyvinylpyrrolidone (PVP), mineral oil (MO) or stearic acid (SA)) copper oxide nanomaterials (CuO NM) (25-55 nm) in a field soil. Soil was spiked at CuO concentrations ranging from 4-1000 mg kg<sup>-1</sup> and used to assess effects on earthworm (*Eisenia andrei*) reproduction. Additionally, soils were sampled over five months to determine the fate of the coated CuO NM through the measurement of available Cu<sup>2+</sup>. Results were compared to previous studies that looked at the fate and effects of uncoated CuO NM and CuSO<sub>4</sub> (as a soluble Cu form). Fewer juvenile *E. andrei* were produced in soils with MO coated CuO (EC50: 37 (6-241) mg kg<sup>-1</sup>) compared to the PVP and SA coated NM, which had a similar response (EC50: 79 (53-118) and 84 (25-283) mg kg<sup>-1</sup>, respectively). Generally, the soils with coated CuO NM had a greater effect on juvenile production compared to the uncoated CuO NM and CuSO<sub>4</sub> spiked soils. The amount of available Cu<sup>2+</sup> measured in the spiked soils could not explain the differences in toxicity observed among the three coatings, suggesting an effect of the coatings or a nano-specific effect. In aged soils, pHs decreased between day 0 and 1 month and the largest increase in measured available Cu<sup>2+</sup> occurred in this first month of aging with the exception of the MO CuO, for which large increases in available Cu<sup>2+</sup> extended to 2 months of aging. After 2 months of aging, the Cu<sup>2+</sup> activity stabilized in all coated CuO NM. The data collected in this study will provide important insights to risk assessors for read-across potential as they evaluate differently coated metallic NM.

#### 1.10.P-We014 Using Novel Multiplexed Algal Cytological Imaging Assay and Machine Learning to Predict the Phytotoxic Mode of Action of LiCoO<sub>2</sub> Nanomaterials to the Green Algae *Raphidocelis subcapitata*

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With copious engineered nanomaterials found in the environment and commerce, it may not be feasible to study them all using conventional toxicological methods. And with a lack of toxicity data, there is a need for more nontargeted, high-throughput profiling assays that can characterize biological activity and predict modes of action (MoA's). In recent years, phenotypic profiling has shown its ability to provide rich sources of data for interrogating biochemical perturbations as the phenotype of a cell is extremely sensitive and strongly influenced by factors such as metabolism, genetic state, and environmental cues. Additionally, it has been shown that specific biochemical perturbations deliver equally as specific phenotypic profiles, and therefore any subset of phenotypic features that deviate from that of healthy cells can serve as a fingerprint to characterize biological activity. Furthermore, when comparing the fingerprint of novel compounds to that of compounds with established MoA's, the probable MoA of these novel compounds can then be identified.

In this experiment, a novel multiplexed algal cytological imaging (MACI) assay was used to investigate the impacts of the mixed metal oxide nanomaterial, LiCoO<sub>2</sub> (LCO), to *Raphidocelis subcapitata*. LCO is largely used as a cathode material in lithium-ion batteries, and with annual productions at high quantities of environmental significance while also lacking infrastructure for recycling and disposal, there is a high potential for environmental release of this material. This is concerning as LCO contains metals with high reactivity and known inherent toxicity. Thus, it will be important to understand how LCO interacts with environmentally relevant organisms, like green algae.

MACI, a high-throughput phenotypic profiling assay, was used to visualize different organelles and obtain a large number of phenotypic features at the resolution of a single-cell. Different sub-cellular structures of *R. subcapitata*, including chloroplasts, neutral lipid droplets, and nuclei, were analyzed in response to LCO exposure. Statistical differences in each structure were observed between treated and non-treated samples, as well as in the overall phenotypic profile, thus exemplifying the ability of MACI to characterize bioactivities in response to nanomaterials. Lastly artificial neural networks were employed to predict the probable phytotoxic MoA of LCO using compounds with established MoA's as a training/reference set.

#### 1.10.P-We015 Compositional Turnover in Macroinvertebrate- and Zooplankton Communities in Response to a Controlled-Release Nano-TiO<sub>2</sub>-Coated Formulation of Carbendazim and its Constituents in Freshwater Mesocosms

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The implementation of nanocarriers is increasingly proposed as a means to reduce pesticide translocation to nontarget sites. The scientific basis to substantiate the intended reduced environmental impact of nanocarrier-based pesticides has however remained marginal to date, and concerns have been raised regarding potential direct- and indirect toxicity associated with the

(unintentional) release of nanocarrier-based pesticides to the environment. We report the findings of a long-term mesocosm experiment in which we assessed impacts of a novel controlled-release pesticide formulation (i.e., nano-TiO<sub>2</sub>-coated carbendazim) and its individual- and combined constituents (i.e., nano-sized TiO<sub>2</sub> and carbendazim) on naturally established freshwater macroinvertebrate and zooplankton communities. Pronounced effects of all applied stressors were observed on a variety of taxonomy- and function-based community parameters, and impacts were most pronounced in carbendazim-including treatments. Our findings demonstrate that 1) when released to nontarget sites, nanocarrier-based pesticides may not necessarily differ in impacts from their active ingredient, and that 2) current environmental concentrations of nTiO<sub>2</sub> may impact zooplankton populations and communities.

#### 1.10.P-We016 Toxicity of the New Emerging Nanomaterials LDH and MoS<sub>2</sub> to Aquatic Organisms: First Results from the Project SCANNER

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SCANNER is a 4-year collaborative project between India and Norway focusing on the development of tools for hazard and risk assessment of new emerging Nanomaterials (NMs). Initially, the partners identified two NMs of interest, Layered double hydroxides (LDH) and Molybdenum disulphide (MoS<sub>2</sub>). The Indian partners were responsible for synthesizing the NMs and supplying them to the partners to be used in *in vitro* and *in vivo* studies to understand the impacts on human health (India) and the environment (Norway). SCANNER aims to conduct controlled exposure experiments to obtain detailed knowledge on the mode of action (MoA) and effects of NMs under realistic environmental conditions (on humans, freshwater (FW) and marine species). Characterisation of behavior of NMs in various exposure media is conducted alongside the various assays. A battery of effect-based tools from cells (both human and fish) to organism level (e.g., copepods, mussels, oysters, chironomids, amphipods, zebra fish embryos (FET)) are being applied to assess MoA and to identify new effect biomarkers. The bioavailability, uptake and transport mechanisms of NMs will also be studied (cell, tissue and organism level). The data generated will be integrated into a hazard and risk assessment approach that can be used for NMs. Results to date indicate little or no observed toxicity at realistic environmental concentrations. Results from the FET tests (with LDH) indicated small effects in survival at concentrations from 1.25-50 mg/L and effects on hatching at 6.25-50 mg/L. No effects were observed at 100 mg/L. For MoS<sub>2</sub> there was no hatching of embryos at 100 mg/L, decreased hatching at 50 mg/L and malformations observed at 1.25mg/L. For the developmental assays with *Tisbe battagliai* exposed to LDH, there were significant developmental delays and mortalities at 25-100 mg/L. For MoS<sub>2</sub> there were mortalities at high concentrations (50 and 100 mg/L) but this may have been caused by a shading effect of the particles or clumping of the algae, limiting the food availability for the developing nauplii. Significant differences were observed between the behavior of MoS<sub>2</sub> in FW media compared to seawater. Based on the results so far, MoS<sub>2</sub> and LDH are not acutely toxic at low concentrations and effects were only observed at concentrations unlikely to be found in the environment. The next stage of the project will focus on lower concentrations of NMs to look at sublethal and chronic effects in the various systems.

#### 1.10.P-We017 Evaluation of the ecotoxic potential of graphene oxide in *Xenopus laevis* tadpoles

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Due to their unique properties, the production of graphene-based nanomaterials (GBMs) including graphene oxide (GO) is growing worldwide to develop new applications. This raises the question of the potential consequences of their release in the aquatic environment. The aim of this work is to assess the ecotoxicological potential of GO using the amphibian, *Xenopus laevis*, as biological model. For this purpose, standardized *in vivo* studies were conducted to evaluate on the one hand the effect of GO on larval growth and related pathways including alterations of the gut microbial communities as well as potential endocrine disruption using respectively 16S sequencing and T3-induced amphibian metamorphosis assay. Larval growth inhibition following exposure to doses ranging from 0.05 to 10 mg/L was shown to be driven by GO surface area while alterations of the gut microbiota structure through an increase of the Firmicutes/Bacteroidetes ratio, a marker of metabolic disorders, might contribute to the observed effects, with no involvement of endocrine disruption. On the other hand, genotoxic potential was assessed through measurement of micronucleated erythrocyte occurrence. Targeted gene expression quantification in qPCR coupled to nanomaterial thermal reduction and characterization allowed to demonstrate which oxygen-containing functional groups are involved in the ROS-mediated induction of genotoxic effects by GO. The obtained results allowed to decipher toxic pathways involved in the physiological alterations of *X. laevis* tadpoles following exposure to GO and to propose a “safer-by-design” approach to mitigate GO toxicity.

#### 1.10.P-We018 Toxicokinetics and Bio-Distribution of Gold Engineered Nanomaterials in the African Sharptooth Catfish

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Nanotechnology provides a promising tool in the manipulation of engineered nanomaterials (ENMs) which can be used for applications in medical treatment. Nanogold (nAu) is used in targeted drug delivery, while gold chloride (HAuCl<sub>4</sub>) is used as a precursor for the synthesis of nAu. The increased use, production, and disposal of nano-enabled products increases the potential risk, particularly to aquatic life. Fish are dependent on aquatic habitats, and water bodies act as a sink for pollutants therefore waterborne exposure is inevitable. Due to the limited knowledge on the absorption, distribution, metabolism, and excretion

(ADME) of nAu, and HAuCl<sub>4</sub> in fish, further investigation is warranted. To determine the tissue-uptake rate of the metal salt and metal ENM a 24 h waterborne exposure to nAu and HAuCl<sub>4</sub>, at 1 mg/L Au each, were independently conducted using adult African sharptooth catfish (*Clarias gariepinus*). Using a systematic approach, the experimental data was used to understand ENM toxicokinetics, crucial to risk assessment. To gain insight into ENM toxicokinetics, a blood-flow limited physiologically based pharmacokinetic (PBPK) model was modified to simulate and compare the ADME of nAu, and HAuCl<sub>4</sub> in catfish. The PBPK model describes the ADME of nAu, and HAuCl<sub>4</sub>, in the gill, air-breathing organ, liver, kidney, and muscle tissues. The model was calibrated using data from both literature such as cardiac output, and experimentation such as organ weights. Chemical-specific parameters were estimated by fitting the model to the experimental data. The model adequately (root mean square < 0.5) predicted the datasets on an organ-level, by simulating the Au ADME in the gill, air-breathing organ, kidney, liver, and muscle tissues, indicative of reliable predictive capability. Simulation results indicate parameters related to ENM-specificity and animal physiology have a profound influence on dose-uptake metrics. Since the physiological effects will depend greatly on the compartmentalization within each specific organ, this study warrants a focus on the cellular and sub-cellular ADME of ENMs in fish. This will permit further model development, and optimization to improve the model's predictive capacity and accuracy.

#### 1.10.P-We019 *In Silico* Prediction of the Nanoparticle – Biomolecule Corona

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The bioactivity, adverse outcomes, and eventual fate of a nanoparticle (NP) in biological environments are known to be mediated through the existence and content of a corona of adsorbed proteins, metabolites, and other small organic molecules acquired by the NP from the surrounding medium. Information about the content of the corona for a given NP in a certain biological environment is therefore key for the prediction of potential toxic outcomes, yet obtaining it remains a highly challenging task due to the necessity to scale up from individual molecules adsorbing within microseconds to the long-term corona formation over the course of hours but also due to extreme diversity of the organic content of biological and environmental liquids. Here, we present a multiscale computational method for the prediction of the NP corona content from first principles without experimental input. We perform atomistic molecular dynamics simulations to obtain interaction potentials between common biomolecular fragments and inorganic NP surfaces and use a machine-learning approach to extend this initial set to a much greater range of small molecules and surfaces, potentially applicable to arbitrary combinations of molecules and surfaces. These potentials are employed in the UnitedAtom (UA) framework to produce orientation-specific adsorption energies of proteins and metabolites to the NP of interest, taking into account the size, shape, surface charge, and chemical composition of the NP. In turn, these adsorption energies are converted to adsorption and desorption rate constants used as input to a random sequential adsorption model of the corona formation. Our methodology takes into account not only the individual affinity of biomolecules towards the NP but also the area occupied by adsorbates and their concentration in the bulk medium, allowing for a more physically realistic model of corona formation. The procedure has recently been validated by comparison to the corona obtained experimentally for silica NPs immersed in a multicomponent protein solution, finding good agreement and confirming the overall validity of our approach.

#### 1.10.P-We020 Metabolomic, Physiological, and Behavioural Responses of the Freshwater Shrimp, *Caridina africana*, Following Exposure to Nanodiamonds and Copper oxide

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Nanomaterials are the building blocks for nanotechnology, which has gained momentum within the science community, especially in the medical field. Examples of such nanomaterials include nanodiamonds and copper oxide. The effects that these two nanoparticles may exert on crustaceans, such as shrimp, are not well documented. *Caridina* is a freshwater crustacean and is a relatively sensitive species, which makes them suitable for ecotoxicity testing. Chronic bioassays were conducted on *Caridina africana* for 96 h. Treatment groups were spiked with sublethal concentrations of nanodiamond – LC<sub>10</sub> and LC<sub>20</sub> (0.98 mg/L and 1.97 mg/L) and copper oxide – LC<sub>10</sub> and LC<sub>20</sub> (0.97 mg/L and 1.94 mg/L). Following 96 h exposure, shrimp from different treatments were randomly selected for metabolic, physiological (heart rate and respiration rate) and behaviour assessment. For the heart and respiration rates, shrimp were either placed under a microscope and a 10-second video was recorded, or in a Loligo respiration chamber to analyze their respiration rate. At least five individuals per treatment were transferred to a 12-well plate and placed in a Noldus Daniovision and behaviour was recorded for 30 min alternating between a light and dark cycle every 5 min. For metabolomics, shrimps were snap frozen in liquid nitrogen. Following extraction, untargeted metabolomic profiles were determined using LC-MSMS techniques. Only the heart rate of copper oxide LC<sub>10</sub> and LC<sub>20</sub> exposures decreased when compared to both the control and the nanodiamond treatment groups. Respiration rate increased with increasing nanodiamond concentrations, but with copper oxide there was an increase in the respiration rate at the LC<sub>10</sub> exposure followed by a decrease at the higher LC<sub>20</sub> concentration. Behaviour responses were more pronounced in the copper oxide exposure groups, and this is accompanied by distinct metabolic profile changes. The effect of copper from the copper oxide nanoparticles is likely the reason for the more negative response of the shrimps to copper oxide materials, while the differences in response to nanodiamonds is attributed to the greater agglomeration rate and physical disturbance effects at the higher exposure concentration.

#### 1.10.P-We021 Evaluation of the Relative Hydrophobicity/Hydrophilicity of Metal and Metal Oxide Nanoparticles

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The impact of hydrophobicity on the fate and toxic potency of nanomaterials is a well-established observation. The hydrophobicity affects most of all the uptake rates of nanoparticles into cells and has a considerable effect on their overall

toxicity. Highly hydrophilic compounds exhibit low cell uptake rates due to their repulsion from the headgroups of the membrane lipids. Highly hydrophobic particles are also poorly uptaken because they are insoluble in the water-based cytoplasm. To be effectively uptaken by the cells, nanoparticles must exhibit a certain hydrophilicity/hydrophobicity balance to be able to cross the membrane and then remain soluble in the cytoplasm. A good measure of the relative hydrophilicity/hydrophobicity is the enthalpy of immersion which is the enthalpy change associated with the immersion of a solute in a liquid. Here we quantify the relative hydrophilicity/hydrophobicity of a nanomaterial as a function of relative enthalpies of immersion of that slab of nanomaterial in water and in octanol, respectively, to represent its behaviour in aqueous and lipid phases. We employed the GROMACS molecular dynamics package to evaluate the immersion enthalpy of 7 metal oxides and of 17 metals samples with three different surfaces each in octanol and in water. Furthermore, we used the force field and unit lattice cell descriptors of each nanomaterial slab as input data to train a neural network model to predict the immersion enthalpy values or a robust ranking thereof. We created an ensemble of neural network models that were trained and validated on various samples of the molecular dynamics dataset. On average, when comparing the calculated immersion enthalpies versus the neural network predicted ones, we obtained correlation coefficients  $R^2$  higher than 0.95 and a Kendall-Tau ranking coefficient higher than 0.9. With the present method, we are able to accurately predict the rankings of various materials' relative hydrophilicity/hydrophobicity using simple molecular interaction and geometric descriptors. Thus, we demonstrate that information on the ranking of materials in terms of their relative hydrophilicity/hydrophobicity can be obtained from first principles using commonly accessible descriptors, thus eliminating the reliance on lengthy molecular dynamics simulations and possibly experimental input.

#### **1.10.P-We022 Considering Matrix Effects in the Assessment of Environmental Safety for Engineered Nanomaterials and Nano-Enabled Products**

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Engineered nanomaterials (ENMs) are often manufactured with a chelating agent to enhance the stability or functionality of the ENMs. This ENM functionality is important to the overall design and manufacture of nano-enabled products (NEPs). In this case study, textiles are coated with silver (Ag) ENMs synthesised with two different chelating agents (quaternized hydroxyethyl cellulose and curcumin) to provide an antibacterial function to the textiles. When considering the NEP safety, the potential ecotoxicological effects of an ENM and NEPs need to be addressed, considering firstly the relative toxicity of the ENMs; secondly, whether toxicity originates from the core particle, the chelating agent or the NEP matrix; and finally, whether there are interactions which may alter toxicity compared to that predicted from the toxicity of the individual components. For soil organisms, exposure to ENMs may occur as a result of direct addition or as constituents of industrial and domestic waste materials and sewage sludge - applied to soil as a conditioner. The aim of this study was to compare the fate and toxicity of Ag ENMs developed as part of a safe-by-design approach for NEPs, to a soil organism in environmentally relevant media. We consider the fate and effects of the ENM together with the chelating agents (alone and in combination) on a model soil species, the annelid *Enchytraeus crypticus*, using acute toxicity tests, with survival monitored through time during the 96h test period. The exposures were performed in media representative of soil pore water (both synthetic and extracted soil pore water). Finally, the overall NEP will be considered by performing tests with coated textile fibres to establish whether differences in the ENMs will finally be important to the overall safety of the NEP.

#### **1.10.P-We023 Multiscale Modelling of Milk Proteins Interaction with Iron Surfaces**

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Understanding interactions occurring between biomolecules and the inorganic materials at the interfaces is important for understanding various phenomena, e.g., formation of protein corona of nanoparticles (NP), fouling of metallic surfaces, biomedical nanoformulations, or in food contact applications (storage, dairy industry, packaging, etc.). Milk fouling and the role of proteins aggregation on heat exchangers and therefore contamination problem has been studied recently to reduce the cost of cleaning and increase the quality of milk products. Herein we present the preliminary results on the study of the interaction of bovine milk proteins with iron metallic surface. Selected metal represents the bulk of one of the most common materials used in the food industry, and bovine milk represents one of the most common sources of proteins in the human diet. By calculating the adsorption energies of the bovine milk proteins with the substrate we quantify the adsorption strength at different conditions and rank proteins by adsorption affinity. The results are expected to correlate with the probability of fouling. Protein adsorption energies were predicted by applying United Atoms (UA) multiscale approach, in which protein and the NP are represented in a coarse-grained (CG) way. The NP is structured as the metallic slab with the uniform density, while the protein is constructed as the rigid body via a "one-bead-per-amino acid" representation. The total interaction of the whole protein with the NP was calculated additively, via individual terms corresponding to each amino acid (AA). The total UA interaction potential between NP and AA is modelled with different resolution at different surface-surface separation distances. This requires parametrisation of the AA-NP potential term at short distances (<1.2 nm) via potential of mean force (PMF) profiles obtained with all-atom AWT-Metadynamics simulations (GROMACS/Plumed). Obtained PMFs were used to predict adsorption energies of the proteins with the UA method at the next step. The coordinates of protein structures (820 in

total) were predicted computationally by i-TASSER software from corresponding sequences.

Obtained information on protein adsorption affinities was further utilized for building a statistical (nanoQSAR/ANN) and adsorption kinetics (AN) model, which can predict protein binding affinities and the corona composition to zero-valent iron surfaces directly from the protein sequence and NP descriptors.

#### **1.10.P-We024 Impact of Storage Conditions on Silver Nanoparticle Stability for Toxicity Studies Using spICP-MS**

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Single particle Inductively Coupled Plasma-Mass Spectrometry (spICP-MS) is an important analytical method for the detection, characterization and quantification of nanomaterials (NMs) in aqueous samples.

SpICP-MS is increasingly being used to study the ecotoxicity and fate of NMs. However, protocols for determining NM concentrations in different media, including tissue, are still being developed. To date, there is little systematic data on how sample storage can impact the characteristics of NMs in aqueous samples. The influence of storage on the quality of NM measurement is important because samples are usually preserved for varying lengths of time and temperature prior to spICP-MS analysis. It is thus unclear how storage conditions affect the quality of NM measurements.

We studied the effect of sample storage conditions using two types of silver nanoparticle (AgNP) suspensions in both MQ water and filtered natural seawater: citrate-stabilized and PVP coated AgNP. We tested the effect of storage duration (1, 7 and 28 days) at four temperatures (21°C, 4°C, -20°C, and -80°C) in the absence of light with spICP-MS.

We observed a strong decrease (approx. -80%) in particle concentration over the storage period for citrate-stabilized AgNP in both MQ and seawater. On the contrary, the concentration of Ag NP in MQ water at 4 and 21°C increased over time (up to +50%). The concentration of PVP-coated AgNP show the same trends, but to a much lesser extent. A slight decrease of particle size over time at all temperatures was observed for citrate-stabilized AgNP suspensions in MQ water. There was however no discernible change in particle size for seawater suspensions over time. The calculated median sizes show a larger standard deviation for the citrate-stabilized particles in seawater than for the particles in MQ water. Also for the PVP coated AgNP no clear trend in change in particle size was observed, regardless of the medium, storage or temperature. Again, the standard deviation for the calculated median sizes of the particles were higher in seawater than in MQ water.

Our data show that the particle concentration of citrate-stabilized AgNP in MQ water is better preserved for up to four weeks when they are stored at 4 or 21°C, but for seawater a storage at -80°C is recommended compared to storage at sub-zero temperatures. The same applies to PVP coated AgNP, which are more stable over time in terms of particle concentration than the citrate-stabilized AgNP.

#### **1.11 Novel Methods and Approaches for Assessing Effluents, Chemicals Toxicity and Surface Water to Support Regulations**

##### **1.11.T-01 DECIDE - An Ecotoxicological Assessment System for Rivers**

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Due to various anthropogenic pressures, surface waters are negatively affected worldwide. In particular, chemical pollution and morphological degradation endanger the ecosystem functions they provide. In Germany, 9.2 % of surface waters achieve the required good ecological status, according to the European Water Framework Directive (WFD). Effective management can counteract the progressive degradation of water bodies and achieve improvements. However, knowledge about the respective main causes on site is indispensable.

In the DECIDE project, an innovative system solution for water management practice is developed as the basis for sustainable water management. For this purpose, an ecotoxicological and WFD-compliant evaluation system will be developed to prioritize water management measures. In practice, the assessment system will help to decide whether chemical pollution or deficient water body structures are the main cause of failing to achieve good ecological status. Effect-based assessment methods are used to determine the "ecotoxicological status class" as an additional parameter.

In 2021 and 2022, the *Hessian Ried* was investigated, which is of great importance as a drinking water resource for the Rhine-Main metropolitan region. At a total of 30 sampling sites, the structural and ecological status was recorded according to existing assessment methods. Ecotoxicological effects were detected on both *in vivo* and *in vitro* levels. After chronic exposure, the snail *Potamopyrgus antipodarum* and the crustacean *Gammarus fossarum* showed significant effects compared to uncontaminated reference sites. At the *in vitro* level, mutagenic, endocrine and dioxin-like effects, as well as baseline toxicity were detected. Furthermore, laboratory and field experiments were performed to detect possible phytotoxic effects. Subsequently, ecotoxicological status classes were determined for all sampling sites. By comparing the ecotoxicological status class with the hydromorphological quality, the contribution of the chemical pollution at a sampling site for failure to achieve the good ecological status can now be estimated more precisely.



### 1.11.T-02 Application of Real-Time Biological Early Warning Systems in Wastewater Treatment Plants: A New Opportunity to Monitor Changing Wastewater Composition?

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Wastewater treatment plants (WWTPs) are a major source of micropollutants to surface waters. Currently, their chemical or biological monitoring is realised by using grab or composite samples, which provides only snapshots of the current wastewater composition. Especially in WWTPs with industrial input, the wastewater composition can be highly variable and a continuous assessment would be advantageous, but very labour and cost intensive. A promising concept is automated real-time biological early warning systems (BEWS), where living organisms are constantly exposed to the water and an alarm is triggered if the organism's responses exceed a harmful threshold of acute toxicity. Currently, BEWS are established for drinking water and surface water but are seldom applied to monitor wastewater. This study demonstrates that a battery of BEWS using algae, water flea, and gammarids can be adapted for wastewater surveillance. For continuous low-maintenance operation, a back-washable membrane filtration system is indispensable for adequate preparation of treated wastewater. Only minor deviations in the reaction of the organisms towards treated and filtered wastewater compared to surface waters were detected. After spiking treated wastewater with two concentrations of the model compounds diuron, chlorpyrifos methyl, and sertraline, the organisms in the different BEWS showed clear responses depending on the respective compound, concentration and mode of action. Immediate effects on photosynthetic activity of algae were detected for diuron exposure, and strong behavioural changes in water flea and gammarids after exposure with chlorpyrifos methyl or sertraline were observed, which triggered automated alarms. Different types of data analysis were applied to extract more information out of the specific behavioural traits, than only provided by the vendors algorithms. To investigate whether behavioural movement changes can be linked to impact other endpoints, the effects on feeding activity of *G. pulex* were evaluated and results indicated significant differences between the exposures. Overall, these findings provide an important basis indicating that BEWS have the potential to act as alarm systems for pollution events in the wastewater sector.

### 1.11.T-03 Considerations for the Use of Behavioral Endpoints and Methods as a Line of Evidence in Regulatory Toxicity Testing

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Regulatory toxicity studies traditionally use endpoints such as mortality, growth, and reproduction. While these traditional endpoints provide reliable evidence in toxicity testing and provide a connection to toxicity effects on populations, interest in use of behavioral endpoints in toxicity testing has been growing. This interest is driven by the sensitivity, technical developments creating increasingly automated frameworks to measure behavior, and the potential to offer evidence on the mode of action of chemical contaminants provided by behavioral toxicity tests. Aquatic organisms have developed specific mechanisms such as identifying prey, avoiding predators, sexual displays, homing behaviors, etc.; all important behaviors in maintaining a functioning ecosystem. Chemicals in the environment can impact aquatic organisms by disrupting or masking these necessary behaviors. Behavioral endpoints can also provide a connection to population level processes depending on the behavioral measures used. These characteristics of behavioral studies can provide supporting lines of evidence when identifying events of toxicity, however the usefulness of these measures is dependent on several factors including the appropriateness of the test organism selected, the behavioral endpoint used, and how the endpoint relates to a population-level effect. This presentation will discuss how current regulations define the use of behavioral endpoints and population level effects and will also explore strategies for using behavioral measures in toxicity testing and provide examples of behavioral toxicity testing data with comparisons to traditional toxicity test measures.

### 1.11.T-04 Effect-Directed Analysis of a Complex Mixture of Polyaromatic Compounds from Contaminated Soil using Bioassays and Zebrafish Embryos (*Danio rerio*)

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In the environment, organisms are exposed to a wide range of pollutants in complex mixtures. Over the last decade, numerous frameworks have been established to consider the potential mixture effects in regulatory decision-making processes. Commonly, the specific composition of complex environmental mixtures is unknown, and one approach to study them is to use effect-directed analysis (EDA), combining fractionation, bioassays, and targeted and non-targeted chemical analysis. Polyaromatic compounds (PACs) are widespread environmental contaminants, that have been detected in high concentrations in environmental samples. Only 16 polycyclic aromatic hydrocarbons (PAHs) are listed as priority pollutants by the US EPA and are regularly monitored. However, more attention has been drawn to the alkylated PAHs (alkyl-PAHs) and heterocyclic aromatic compounds (NSO-PACs) since they can be more toxic than the parent PAHs. In the present study, zebrafish embryos and *in vitro* bioassays were used to investigate the behavioral and molecular effects of an extract from a soil sample collected at a former gasworks site in Sweden. Four different CALUX® assays were used to evaluate aryl hydrocarbon receptor (AhR) activation (DR-CALUX and PAH-CALUX), estrogen receptor activation (ERα-CALUX), and androgen receptor inhibition (anti-AR-CALUX) of the soil extract. The zebrafish behavior was measured as larvae movement during exposure to interchanging light and dark periods. In addition,

the response to mechanical stimuli was recorded using a tapping device. Quantitative gene expression analyses were conducted on a set of 36 selected genes that are known to be directly linked to certain adverse outcomes. The complex mixture caused hyperactivity in darkness and hypoactivity in light. The larvae were also hypoactive after the mechanical stimuli. The qPCR analysis showed that 15 genes were differentially regulated. For instance, one gene connected to eye pigmentation (*opn1sw1*) was downregulated and a gene involved in heart failure (*fpgs*) was upregulated. In addition, the extract gave a significant response in three bioassays (ERα-, DR-, and PAH-CALUX). To identify drivers of the toxicity the extract was fractionated, and thirteen out of 96 fractions were active at 120 hpf. The observed effects were mortality, lack of blood circulation, unhatched embryos, and edemas. In the last step of this study, the compounds in the active fractions will be identified.

#### **1.11.T-05 Combined Use of Transcriptomics and Proteomics as an Evaluation Tool of Environmental Toxicity in a Beluga Whale (*Delphinapterus leucas*) Population Highly Exposed to Contaminants**

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The St. Lawrence Estuary (SLE) beluga whale (*Delphinapterus leucas*) population in Canada is endangered. It has been postulated that exposure to elevated concentrations of polychlorinated biphenyls (PCBs), chlorinated pesticides, polybrominated diphenyl ethers (PBDEs), and emerging halogenated flame retardants (HFR) might be contributing to the decline of this population. Recently, concentrations of PCBs, chlorinated pesticides, and emerging HFRs in SLE male beluga blubber were shown to correlate with skin transcript levels of genes coding for nuclear receptors and proteins involved in the regulation of thyroid and steroid hormones as well as the metabolism of xenobiotics. A growing number of studies investigating contaminant exposure-related effects in cetaceans rely on “omics” methods such as transcriptomics or proteomics, as these approaches require small amounts of tissue and provide a maximum of biological information. To improve our understanding of the impacts of contaminant exposure on the health of SLE belugas and the mechanisms of toxicity involved, we compared transcriptomic and proteomic skin profiles between adult male belugas from the SLE and a reference population (Eastern Beaufort Sea, Canada). Being exposed to dramatically different levels of contaminants the evaluation of transcript and protein profiles will allow to shed some light on specific genes, proteins and pathways affected by the exposure to organic contaminants. Total RNAs and proteins were isolated from skin samples of adult male belugas from the SLE ( $n = 39$ ) and Eastern Beaufort Sea ( $n = 34$ ). Skin was also used to determine carbon and nitrogen stable isotope ratios, and PCBs, PBDEs and emerging flame retardants were measured in blubber. RNA sequencing and proteomics analysis allowed the identification of 21 646 unique gene transcripts and 6152 proteins. A total of 3676 genes and 128 proteins were upregulated, and 1418 genes and 113 proteins were downregulated in SLE belugas compared to the reference population. Ongoing work will characterize the functional profile of differentially expressed genes and proteins to understand the biological implications of contaminant exposure, and potential biomarkers of contaminant exposure will be suggested. The combined use of transcriptomics and proteomics is a promising tool for wildlife ecotoxicology research and has the potential to significantly improve the risk evaluation of organic contaminant exposure in wildlife populations.

#### **1.11.P Novel Methods and Approaches for Assessing Effluents, Chemicals Toxicity and Surface Water to Support Regulations**

##### **1.11.P-Tu032 Predicting the Impacts of Chemical Pollutants on Animal Groups**

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Chemical pollution is among the fastest-growing agents of global change. Synthetic chemicals with diverse modes-of-action are being detected in the tissues of wildlife and pervade entire food webs. Although such pollutants can elicit a range of sublethal effects on individual organisms, research on how chemical pollutants affect animal groups is severely lacking. This is a critical oversight because many animals engage in social interactions over their lifetime and live within highly structured societies or form loosely structured social groups. These animals coordinate their behaviours with others to provide protection against predation, gain reproductive opportunities, find food, and reduce energy expenditure. Here, we present a framework that outlines how chemical contaminants could disrupt behavioural processes that are instrumental in the emergence and self-organisation of animal groups. We provide a roadmap for prioritising the study of chemical pollutants within the context of sociality and highlight important methodological advancements for future research. Integrating animal sociality and collective behaviour into ecotoxicity studies is particularly important in light of recent evidence that behavioural endpoints are largely ignored in chemical risk assessments when not linked to population or higher-order ecological outcomes. Thus, our framework provides an important guide for researchers and practitioners to predict how chemical stressors will likely affect the emergence, organisation, and function of animal social groups.

##### **1.11.P-Tu033 A Rapid Approach to Characterise the Photosynthetic Efficiency and Growth Inhibition of a Marine Microalgae Exposed to Metal Mixtures**

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Marine algae are under threat from increased chemical (including metals) and nutrient run-off. Due to their trophic position and

need to utilise trace metals for biochemical processes, microalgae are often considered as the first target of metal toxicity. To better inform risk assessments and develop more holistic impact considerations, combined toxicity assessment to characterise the joint effects of mixtures is needed. However, mixture toxicity studies can be difficult to conduct, with resource, time, and financial limitations. These can be mitigated with a Design of Experiments approach, including Definitive Screening Design (DSD), which can determine main effect drivers and second-order effects ( $P < 0.05$ ). Using DSD and rapid stress assessments, such as pulse amplitude modulated fluorometry (PAM), could be useful in combined toxicity assessments with microalgae, macroalgae and plants. Using the microalgae *Tetraselmis suecica*, this study conducted a rapid combined toxicity assessment for the metals copper, nickel, and zinc. Growth was measured over 7 days of exposure, alongside the impacts on photosynthetic performance using an Imaging PAM. IPAM samples were dark adapted and parameters measured, including photochemical (qP) and non-photochemical quenching (qN and NPQ), effective photosystem II quantum yield (YII) and electron transfer rate (ETR). By using a rapid assessment tool to measure photosynthetic stress and a DSD mixture design, we found that metal mixtures affected algal photosynthesis photochemistry and inhibited growth. All three metals were identified as main effect drivers reducing *T. suecica* growth, with metal interactions between all metal combinations. The  $F_v/F_m$  fluorescence analysis demonstrated that copper was the main effect driver and there were no interactions with the other metals. For light acclimated parameters, copper was the only main effect driver within the mixture for qN, NPQ, qP, YII and ETR, with interactions between copper with nickel and zinc for YII and ETR. These trends in chlorophyll fluorescence parameters suggests that the light harvesting capacity of PSII was downregulated during metal exposure and photoinhibition was occurring, in turn limiting photosynthetic efficiency and growth rates. As most mixture studies focus on the adverse effects (e.g., growth), this study provides evidence for and a demonstration of how DSD can be used to enhance mechanistic understanding of combined toxicity of metals in microalgae.

#### 1.11.P-Tu034 Standardization of a Short-Term Chronic Method using *Daphnia magna*

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In 2009, US EPA published development and validation of a *Daphnia magna* 4-d survival and growth test method in ET&C. This method is now being standardized so that it can potentially be used as another short-term chronic test in the National Discharge Elimination System (NPDES) permitting process for whole effluent toxicity testing and other regulatory assessments. Two ORD laboratories (Duluth, MN; Cincinnati, OH) have conducted additional testing to optimize method performance, followed by 4-d standardization studies to evaluate method variability and reproducibility using reference toxicants. The standardization studies consisted of triplicate tests conducted by both laboratories, with three toxicants: ammonia, zinc, and diazinon. Each toxicant was also tested using a 7-d chronic *Ceriodaphnia dubia* test for comparison. For ammonia, intra-laboratory variation in IC25 values for *D. magna* weight was relatively low (CV = 17%, 27%, for Duluth and Cincinnati, respectively), but there was an apparent difference in toxicant sensitivity between laboratories, with IC25 values of 24 to 33 mg/L as N for Duluth, and from 49 to 84 mg/L as N for Cincinnati. For diazinon, CVs were also low = 25% and 7% (Cincinnati and Duluth, respectively), but the IC25 values were higher for Duluth (0.63-1.01 µg/L) than Cincinnati (0.38-0.43 µg/L). *C. dubia* results for diazinon were similar for both labs (0.20 and 0.17 µg/L). For zinc, Duluth's IC25s ranged from 78.8-114 µg/L while Cincinnati's two tests were 110 & 143 µg/L. *C. dubia* results for zinc were 38 µg/L for Duluth and 103 µg/L for Cincinnati. Reasons for the differences between the labs in apparent sensitivity are under investigation, but may be due to differences in test solution pH or potential differences in culture methods, stock genetics and algal food preparation procedures. A *post hoc* power analysis was done to quantify the relationship between number of replicates and statistical power to detect differences between experimental treatments. Future method standardization work will include evaluation of whole effluents with both species.

#### 1.11.P-Tu035 *Hydra viridissima* as Model to Study the Toxicity of Environmental Contaminants: A Case Study with Metals

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Anthropogenic activities are globally increasing the concentration of contaminants such as metals in the environment, particularly in the freshwater environment, thus leading to significant effects at population, community, and ecosystem levels. Therefore, cost-effective tools for continuous monitoring of these contaminants must be pursued. In this sense, hydras can be a valuable biological model that may provide rapid information on the toxicity of a substance/particle, easily recognizable by potential morphological and behavioural changes. Accordingly, the present study aimed to assess the toxicity of three metals (copper, zinc, and cadmium) focusing on lethality, morphological changes, and feeding rate of the freshwater cnidarian *Hydra viridissima* after a 96-h exposure period. A set of metal concentrations (copper 0.09-1 mg/L, zinc 0.24-2.77 mg/L, and cadmium 0.14-1.57 mg/L) was prepared by successive dilutions of the metals' stock solutions in hydra medium. Organisms were checked every 24 h for mortality and morphological alterations. The feeding behaviour was also assessed after 96 h exposure. The lethality data allowed the estimation of the median lethal concentrations (96 h-LC<sub>50</sub>) which allow the toxicity ranking as copper > cadmium > zinc. *H. viridissima* mortality was detected after the 96-h exposure period at concentrations equal or higher than 0.667 mg/L, reaching 100% at the highest concentrations tested for each metal. Regarding malformations, more than half of the organisms presented shortened body and/or tentacles. In the post-exposure feeding assay, hydras exposed to intermediate concentrations (0.138 and

0.207 mg Cd/L; 0.243 and 0.364 mg Zn/L) displayed a trend to ingest more prey than controls, although only significantly in the zinc treatments. Overall, *H. viridissima* sensitivity to metal exposure strongly supports its use in the assessment of waterborne contaminants based on a wide range of endpoints. In addition, deriving up to date toxicity values based on these organisms may be important for setting more conservative benchmarks.

#### 1.11.P-Tu036 Environmental Levels of Carbaryl Impair Zebrafish Larvae Behavior: The Potential Role of ADRA2B and HTR2B

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The insecticide carbaryl is commonly found in nondirectly exposed freshwater ecosystems at ng/L concentrations, below its NOEC for acetylcholinesterase (AChE) inhibition reported for different fish species. Therefore, environmental levels of carbaryl are commonly considered safe for fish communities. In this study, we showed that after only 24 h of exposure to environmental concentrations of carbaryl (0.066-660 ng/L), zebrafish larvae exhibit impairments in essential behaviors. Interestingly, the observed behavioral effects induced by carbaryl were acetylcholinesterase-independent. To elucidate the molecular initiating event that resulted in the observed behavioral effects, *in silico* predictions were followed by *in vitro* validation. We identified two target proteins that potentially interacted with carbaryl, the  $\alpha$ 2B adrenoceptor (ADRA2B) and the serotonin 2B receptor (HTR2B). Using a pharmacological approach, we then tested the hypothesis that carbaryl had antagonistic interactions with both receptors. Similar to yohimbine and SB204741, which are prototypic antagonists of ADRA2B and HTR2B, respectively, carbaryl increased the heart rate of zebrafish larvae. When we compared the behavioral effects of a 24-h exposure to these pharmacological antagonists with those of carbaryl, a high degree of similarity was found. These results strongly suggest that antagonism of both ADRA2B and HTR2B is the molecular initiating event leading to the observed behavioral effects in zebrafish larvae exposed for 24 h to environmentally relevant levels of carbaryl.

#### 1.11.P-Tu037 Pull-Down Assay as a Novel Approach for the Identification of Compounds Interfering with Thyroid Hormone Signalling in Complex Environmental Mixtures

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Plasma protein transthyretin (TTR) plays an important role in transport of thyroid hormones (T3 and T4) in vertebrates. Some environmental pollutants can compete with natural TTR ligands, which may disrupt TTR binding and thyroid hormone transport and lead to neuro/developmental and metabolic disorders. Little is known regarding the ability of chemicals occurring in aquatic environment to bind to the TTR protein. Our results indicate that complex environmental mixtures associated with discharges from waste-water treatment plants can elicit such effect, but the effect drivers are unknown.

Here, we present one of the solutions of this problem, a novel method based on an interaction of the TTR with its ligands, called pull-down assay. Recombinant TTR is prepared by inserting TTR sequence into plasmid and expressed in *E. coli*. The purified recombinant TTR is incubated with prioritized extracts of environmental water samples (EwS), leading to the potential binding of TTR ligands to TTR protein. The complex is bound to magnetic Ni<sup>2+</sup> affinity resin via Histidine tag added into TTR sequence. Magnetic particles with bound protein-ligand complex are attached to the surface of the reaction tube using a magnetic holder and the washing steps lead to the displacement of unbound chemicals. The complex is then eluted with an imidazole solution. TTR is denatured with solvent with the ligands released into the solution. The identification of the ligands is performed via non-target MS analysis on LC-Orbitrap and confirmed with target MS analysis. The potential ligands can be identified using library searches and are further tested for their activity in TTR binding bioassay. Finally, the contribution of the identified compounds (e.g., diclofenac and its metabolites) to previously reported endocrine-disrupting potency (TTR binding) of the EwS can be characterized.

#### 1.11.P-Tu038 The Use of Heart Rate Monitors to Determine the Effects of Environmental Stressors in the Common Shore Crab (*Carcinus Maenas*)

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Wearable technologies have advanced substantially in the human fitness and health field and offer huge potential for the monitoring of wildlife. In this study, we aimed to determine the utility of wearable fitness watches as a noninvasive method to monitor the health of crabs. Traditional methods examining the physiology of decapod crustaceans have used wired approaches which are invasive and logically challenging for environmental toxicology. The objective of the study was to determine whether over-the-counter fitness watches could be utilised to monitor heart rate in a decapod crustacean (Common shore crab, *Carcinus maenas*). The accuracy and reliability of these devices are being explored through a series of ongoing experiments using temperature and cardio stimulants. Results so far indicate that fitness watches can detect crab heartbeats through the carapace at

rates equivalent to former studies with some clear peaks through handling stress. While there is still considerable optimisation required and the determination of baseline data, these preliminary results point huge potential in this approach to in vivo studies in the laboratory or in situ monitoring in the environment.

#### **1.11.P-Tu039 Sperm Quality Assessment in *Ficopomatus enigmaticus* (Fauvel, 1923): Effects of Selected Organic and Inorganic Chemicals Across Salinity Levels**

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Contamination by organic and inorganic compounds remains one of the most complex problems in both brackish and marine environments, causing potential implications for the reproductive success and survival of several broadcast spawners. Particularly, aquatic contamination by trace elements, polycyclic aromatic hydrocarbons (PAHs), and surfactants is known to be of critical environmental concern due to their high environmental concentrations, persistence and toxicity in different organisms.

*Ficopomatus enigmaticus* is a filter-feeding tubeworm polychaete, well adapted to brackish water conditions, for which the reliability to be used as a candidate model organism for ecotoxicological purposes has been already described. Although most studies were focused on polychaete juvenile and adults, few have been devoted to compounds effects on sperms. Spermotoxic impact caused by environmental pollutants could lead to transmission of damage to offspring and, in turn, potential implications for reproductive capacity and population preservation. Therefore, the present study investigated the putative impact of five trace elements (zinc, copper, cadmium, arsenic and lead), one surfactant (sodium dodecyl sulfate, SDS) and one PAH (benzo(a)pyrene) on *F. enigmaticus* sperm quality across salinity variations. Sperms were exposed *in vitro* to different contaminant concentrations under four salinity levels and effects on sperm function were assessed in terms of oxidative stress, membrane integrity, viability, and DNA damage. Results showed that sperm impairments induced by organic contaminants were more evident than those induced by inorganic ones, with the largest effects exerted by SDS. When both stressors act in combination, variations in biomarker responses were observed. Specifically, due to salinity influence on physio-chemical speciation of inorganic compounds, major impairments were detected at the higher and lower salinity levels. With respect to organic compounds, because of direct interference of salinity on contaminant toxicity, higher salinity levels enhance their concentration-dependent effects. Overall, the high tolerance showed by *F. enigmaticus* sperms to salinity variations confirms the use of this species as promising organism for an early assessment of marine/brackish water pollution. The present study provides the effectiveness of fluorometric/photometric methods as integrative or alternative endpoints to investigate the spermotoxicity potential of pollutants.

#### **1.11.P-Tu040 Effect-Based Monitoring Tools Reveal that Chemical Pollution Counteracts the Restoration Success of Streams**

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In 2027, the third planning cycle of the European Union Water Framework Directive (WFD) ends and still more than 90 % of German surface waters do not meet its objectives. The main drivers causing degradation of aquatic ecosystems are the presence of contaminants, morphological degradation and high nutrient loading. However, current monitoring methods make it difficult to predict if the poor status of water bodies is due to chemical pollution or morphological degradation. Moreover, recent studies have shown that in rivers with a high proportion of treated wastewater, hydromorphological restoration was not only unsuccessful in improving ecological status, but that the restored sections had higher toxicities than the unrestored reference sections of the same river. Effect-based methods (EBMs) can function as scientifically sound and target-oriented monitoring tools to get a better insight into the pollutants and mechanisms underlying the observed effects. Given this background, the present study aims to use EBMs at organismic and sub-organismic levels to better assess five restored streams and their associated non-restored reference site in Hesse (Germany). We aim to determine whether water pollution or structural deficits are the main causes of the failure to achieve good ecological status.

For this purpose, we performed various *in vitro* tests on water and sediment samples, such as recombinant yeast reporter gene assays for estrogenic, androgenic and dioxin-like activity, Microtox assay for baseline toxicity, Ames fluctuation assay for mutagenicity and AREc32 test for oxidative stress response. Additionally, *in vivo* data on reproductive toxicity using *Potamopyrgus antipodarum* were obtained and further *in vivo* testing using *Hyalella azteca* and fish embryos (*Danio rerio*).

Considering the age of the restoration measures carried out on our sampling sites (approx. 20 years old), we did not expect strong differences between the non-restored reference site and the restored site. Applied EBMs revealed toxic activities in water and sediment samples. So far, the preliminary results do not indicate a clear correlation between toxicity and the morphological condition of the sampling sites. Accordingly, the present findings suggest that the pollutant load counteracts the restoration success. In future studies, more recent restoration projects (approx. 2-5 years old) will be investigated expecting to find higher toxicity in restored river sections.

#### **1.11.P-Tu041 Is Olive Oil Value Chain an Environmental Risk? Ecological Quality Evaluation of Tua River (Portugal) and Multitrophic Toxicity Assessment**

**Silvana Teixeira Costa<sup>1</sup>, Fernando Miranda<sup>2</sup>, Fernando Teixeira<sup>2</sup>, Aya Zidouh<sup>2</sup>, Conceição Fernandes<sup>3</sup>, Luis Dias<sup>3</sup>, Amílcar**

Teixeira<sup>3</sup> and Zulimar Hernandez<sup>1</sup>, (1)MORE- Laboratório Colaborativo Montanhas de Investigação, Portugal, (2)Instituto Politécnico de Bragança, Portugal, (3)CIMO - Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Portugal Portugal is amongst the main producers of olive oil worldwide, where Trás-os-Montes e Alto Douro region is the second national producer. The last two decades were marked by the technological transition in the olive oil industry, responsible for the generation of a by-product called olive pomace (>75% water content). Currently, almost all olive pomace generated in this region is processed in a single outdated industry for the extraction of oil for further refining and sold as edible oil. During this process, olive mill wastewaters (OMWW) are produced, which are stored in high volume and open-air lagoons. Although it is known that olive pomace is phytotoxic due to its high organic load, deeper knowledge is necessary on how OMWW can impact habitat's integrity, namely freshwater ecosystems.

Therefore, eight sampling sites along Tua River (Douro basin) and tributaries were seasonally monitored for a year (2021-2022), following standardized procedures defined by the Water Framework Directive. Environmental data were registered, considering water physicochemical (common metrics and specific pollutants), hydromorphological (River Habitat Survey) and biological (fish and invertebrates) elements. Moreover, to understand the toxicant role of OMWW, crustacean *Daphnia magna* and mussel *Unio delphinus* were chosen as bioindicators organisms. Organisms were used to perform acute and chronic exposures, respectively. Assessments were performed following standardized procedures (24 h Immobilisation test, metabolic capacity, antioxidant responses -catalase and superoxide dismutase- and lipid peroxidation).

When comparing the obtained results, sites located downstream of oil extraction industries showed a significant decrease on the water quality and aquatic habitats. Several ecological metrics and multivariate analyses detected the ecological impact of the effluent. Additionally, the conducted 24 h LD50 assays revealed that the doses required to cause the death, of at least 50% of the tested population, is equivalent to 0.873% (v/v) of OMWW. The increase of reactive oxygen species may have triggered the antioxidant enzymes that, though ineffective, led to increased metabolic rates.

The present research highlights the impact of olive industry upon surrounding ecosystems and the importance to develop ecologic solutions to treat OMWW, reducing its toxicity and, therefore, promoting biodiversity conservation.

#### **1.11.P-Tu042 Ecotoxicological Evaluation of Effluents Originated by the University Hospital, UFSC , Florianópolis – SC, BRAZIL.**

**Carlos H.L. Soares<sup>1</sup>**, Ivana Eunice Baptista<sup>2</sup>, Claus T Pich<sup>2</sup> and Mateus V Machado<sup>2</sup>, (1)Biochemistry, Universidade Federal de Santa Catarina, Brazil, (2)Biochemistry, Federal University of Santa Catarina, Brazil

Hospital effluents are considered potentially hazardous pollutants. Thus, we sought to characterize the effluent from the University hospital in the city of Florianópolis-SC, through physical-chemical and ecotoxicological tests. Physico-chemical analyzes of the parameters ammonia, nitrate, phosphate, total phenols and gas chromatography, and acute ecotoxicological tests in different concentrations with organisms of different trophic levels (*S. subspicatus*, *D. magna*, *Artemia* sp., *D. rerio*, *L. sativa*, and *A. cepa*). Characteristics of fresh sewage were identified through chromatography and relationships between the concentrations found in the physical-chemical tests and the behaviors of the models tested in the bioassays. In the acute tests, stimulation of the algal growth rate was found in most of the concentrations tested with *S. subspicatus* and in some of the tested points, interference from the low levels of phosphate where there was no growth and the high level of nitrate and ammonia where inhibition occurred. Total growth of *L. sativa* was stimulated in most concentrations and where there was no growth, ammonia values were high. Inhibition of apical growth of *A. cepa* roots was observed in most concentrations; however where nitrate levels were lower, the roots grew equal to the control. Toxicity to *Artemia* sp. mainly in concentrations where the ammonia levels were higher. Mortalities, deformations and morphological delays occurred in the models of *D. rerio*, where the values of ammonia and nitrate were higher, the mortalities were also higher. No changes or mortality were recorded in the concentrations tested with *D. magna*, whose model is one of the main test organisms used as a reference in current Brazilian legislation.

#### **1.11.P-Tu043 Physicochemical and Toxicological Characterization of Samples from Water Sources Neighboring Rice Plantations**

**Carlos H.L. Soares<sup>1</sup>** and Ivana Eunice Baptista<sup>2</sup>, (1)Biochemistry, Universidade Federal de Santa Catarina, Brazil, (2)Biochemistry, Federal University of Santa Catarina, Brazil

The southern region of Brazil is dominated by wetlands, which are attractive ecosystems for irrigated rice production and accounted for 68% of national rice production in 2021. This type of farming is generally practiced in the vicinity of water sources, and requires intensive use of chemical inputs resulting in serious risk of water contamination. For these studies were chosen two sites in Cachoeira do Sul river (n° 3 and 4), from which the water is pumped to the management of the rice fields. By recycling, these sites get back the water and wastes. Next to these two sites in the same river, another site was sampled (No. 5 ). In this case it is directly in contact with the culture of rice through a pale of communication. To define a possible site to be used as a reference site, without contamination, another sampling site (No. 7 - Table 1) was chosen for its crystal clear water, thus without the turbidity of the others, caused by the aforementioned recirculation. This site has no contact with the rice fields. A water sample was also taken directly from a framework of growing rice (No. 1 - Table 1) in a nearby area, however, without direct communication with the sites mentioned above. The samples were collected in November 2008, during the rice cultivation period and therefore the application of pesticides on crops. Ecotoxicological tests were performed using a microalgae (*Scenedesmus subspicatus*), microcrustacean (*Daphnia magna*) and *Danio rerio* embryos. Analyses of water samples were performed by GC/MS. The results obtained showed medium-high toxicity for microalgae and microcrustaceans and slight toxicity for *Danio*

rerio embryos. Chromatographic analyses detected the presence of bentazone, as the main herbicide, in concentrations of 150 µg – 450 µg, glyphosate, 80 µg – 110 µg, carbofuran 20 µg to 60 µg, and the insecticide quinalphos, 110 µg.

#### 1.11.P-Tu044 Haloacetonitriles React with Proteins via Three Distinct Reaction Pathways

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Disinfection by-products (DBPs) are formed in disinfected/sanitized water which have been correlated with incidences of disease such as bladder cancer and adverse pregnancy outcomes. Disinfection by-products are a large portion of the human exposome and thus are prioritized for human health regulations. There are >700 DBPs discovered, however the DBP drivers of toxicity and their mechanisms of action within the cell are unknown. Recently, haloacetonitriles (HANs) have been highlighted as an emerging DBP with stronger toxicity than regulated DBPs. This talk will elucidate the toxicity mechanism of HANs, including monoHANs and polyHANs, by investigating their reactivity with human proteome. Unexpectedly, despite the 'simple' structures of HANs, three distinct reaction pathways were observed which led to differing toxicity of HANs depending on the type and number of halogens. Both substitution and addition reaction pathways will be discussed, the latter of which is a novel pathway never reported for DBPs. Cytotoxicity and oxidative stress bioassays were employed to determine the ability of various HANs to induce toxic effects and the role of each HAN's preferential reaction pathway in predicting harmful effects in the human cell. Activity based protein profiling (ABPP) was used to image *in vitro* HAN adduction effects on protein thiols in human cells. Finally, this project involves the use of OMICs by incubating HANs *in vitro* in human embryonic kidney (HEK293) cells which are pertinent to the exposure of DBPs. Proteomics will be used to elucidate the specific HAN-attacked proteins and the dominant HAN reaction mechanism *in vitro* thus elucidating the dominant pathway reaction pathway and prioritizing HANs using their chemical reactivity. This talk will reveal the underlying chemical pathways in which HANs react with thiol proteins and the importance of fundamental chemistry in assessing water toxicity.

#### 1.11.P-Tu045 Using Tissue Residue-Based Effects Concentrations in Setting Sediment Guidelines for Dioxin-Like Compounds

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Setting sediment guidelines for chemicals that bioaccumulate, such as dioxin-like chemicals, can be problematic. Guidance for deriving such guidelines encourages the use of sediment toxicity tests or the use of field data; however, these methods do not work well with chemical groups like the dioxin-like compounds.

Sediment ecotoxicity data are almost nonexistent for dioxin-like compounds. Field data predominantly address effects on invertebrates which are not the most sensitive organisms for this chemical group. Research to understand the ecotoxicity of this group of chemicals for aquatic organisms has identified that the most sensitive organism type is fish – particularly the early life stages and effects concentrations based on tissue residues provide more robust endpoints.

To assess the suitability of these sediments for ocean disposal, a defensible sediment quality guideline value (SQGV) for dioxin-like compounds, expressed as pg toxic equivalent (TEQ)/fish/g dry weight, was required and work was undertaken to review the basis of existing guidelines.

The findings showed that there were too many uncertainties associated with a value derived using effects data from field studies for this chemical group. A similar issue was associated with values based on equilibrium partitioning from sediment to pore water, largely associated with the wide range of reported sediment:water partition coefficients.

A tissue residue approach was identified based on equilibrium partitioning between sediment and organisms determined using tissue concentrations in fish, the most sensitive aquatic biota, and biota: sediment accumulation factors. The calculation of an appropriate SQGV used data for dioxin-like compounds in both fish and sediments from Sydney Harbor. A conservative SQGV for dioxin-like compounds of 70 pg TEQ/g dry weight was deemed to be adequately protective of biota that might be exposed to these contaminants in sediments.

#### 1.11.P-Tu046 Role of Alkylated Polycyclic Aromatic Hydrocarbons in Mixture Toxicity from a Legacy Creosote Site

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Creosote is a pesticide used to preserve wood products. Derived from distillation of coal tar, creosote is a complex mixture containing mostly polycyclic aromatic hydrocarbons (PAHs) and their derivatives. Alkylated PAHs are abundant constituents of creosote and many petroleum products and are known to become enriched relative to their respective parents through weathering. Less is known about the toxicity of alkylated PAHs than their parent compounds. Despite this, alkylated PAHs have been shown to contribute substantially to the toxicity of PAH mixtures in the environment. The goal of this study is to understand the contribution of alkylated PAHs to the toxicity of a complex, weathered mixture from a legacy creosote site. This study utilizes low density polyethylene passive samplers deployed at a former wood treatment facility to accumulate freely dissolved organics in the surface water. Passive sampler extracts are analyzed by gas chromatography - tandem mass spectrometry for parent and alkylated PAHs. To assess toxicity, embryonic zebrafish are exposed to passive sampler extracts in 96-well plates and are observed at 24 and 120 h postfertilization for a suite of behavioral and morphological endpoints. A year-long sampling campaign at the site has shown substantial variability in chemical abundance and toxicity with sum PAH concentrations in two adjacent



months varying by greater than a factor of two and LC50 values varying by a similar magnitude. Alkylated PAHs constituted the majority of measured PAHs in all samples (83-89%). Ongoing work seeks to determine the drivers of toxicity and the role of alkylated PAHs by fractionation of field collected mixtures through effect-directed analysis. Fractionation by gel permeation chromatography revealed that three fractions together recapitulated the toxicity of the whole mixture. The fraction containing alkylated PAHs caused the majority of the toxicity while the fraction containing many unsubstituted PAHs caused less than 10% mortality. A third unidentified fraction caused high incidence of notochord malformation in zebrafish at low concentrations. While further toxicant confirmation is necessary, these results suggest that highly abundant alkylated naphthalenes and phenanthrenes are responsible for driving toxicity rather than the routinely monitored parent PAHs. Understanding the role of alkylated PAHs can inform remediation efforts and improve our ability to protect human health and water quality.

#### 1.11.P-Tu047 Estrogenic Effects on Wastewater Receiving Bodies; an EDA Approach Using YES Bioassays in the Basque Country

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The scientific community is recently evaluating the fate and distribution of contaminants of emerging concern in aquatic ecosystems receiving wastewater treatment plants (WWTP) effluents. Those latter accumulate housing and industrial wastewaters and are not always able to degrade compounds such as personal care products and pharmaceuticals (PCPPs) or consumption chemicals (additives, detergents, PFAS, plasticizers, etc.) which are delivered into rivers and marine waters with an unpredictable effect in the aquatic ecosystems. Among the undesired effects in wild biota, endocrine disruption is of particular concern. Within this context, an effect-directed analysis approach (EDA) was implemented in the Urola river basin in the Basque Country, north Spain. It is impacted by the Zuringoain WWTP that gathers wastewater from 3 municipalities and the Zumarraga hospital, being the hospital water the 2 % of the total river flow during summer season. Two concurrent high volume samples (50 L) of the WWTP effluent and the river area where the effluent impacts were taken in low water period (Sept 2022) using a large volume solid-phase extraction device loaded with a triphasic cartridge (Chromabond HR-X (Macherey-Nagel), Sepra ZT-WAX and Sepra ZT-WCX (Phenomenex)). Both were fractionated by means of preparative chromatography onto a C18 column into 18 sub fractions, and tested for potential estrogenic activity using the A-YES (yeast estrogenic screening) bioassay. Estrogenic fractions will be further fractionated onto an aminopropyl column and subsequently biotested. A suspect screening approach will be used for the tentative identification of the isolated compounds in the active fractions with their confirmation via target analysis (~300 compounds) on a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive Focus Orbitrap MS for xenobiotics. Moreover, an UHPLC system Agilent 1290 Infinity II coupled to an Agilent 6430 triple quadrupole mass spectrometer will be used for the target analysis of 16 hormones.

The comparison of the identified compounds in the river and in WWTP effluent will point to the missing information regarding the potential estrogenic chemicals and their fate and relevancy once delivered in the basin.

#### 1.11.V Novel Methods and Approaches for Assessing Effluents, Chemicals Toxicity and Surface Water to Support Regulations

##### 1.11.V-01 Assessment of Behavioral Responses in the Freshwater Mussel *Sinanodonta woodiana* to Exposure to Six Model Contaminants

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Behavioral biomarkers are considered sensitive tools to assess the impact of contaminants in organisms in a direct and ecologically relevant way, yet the use of such markers in freshwater mussels is still in its infancy. High frequency noninvasive (HFNI) valvometers on mussels are a good example for an application of such biosensors which allow continuous monitoring of gaping and filtration behavior. Due to their sedentary lifestyle and as filter-feeders, mussels are specifically sensitive to changes in the ambient water matrix and are therefore ideal sentinels for potentially harmful conditions.

For the application of such biosensors it is necessary to assess potential responses to different substance classes and to interpret which behavioral patterns are meaningful for the determination of sensitivity thresholds. Therefore, we examined the sensitivity of mussels' valve gaping behavior towards six selected contaminants in the ambient water by simulating pulse exposure scenarios in a laboratory setup.

As model stressors, we selected substances with different modes of action, namely microplastics, salt, copper, diclofenac,  $\alpha$ -cypermethrin, and phenanthrene. Reactions of the freshwater mussel *Sinanodonta woodiana* ( $n_{\text{total}} = 94$ ) were recorded within 1 week experiments using Hall sensor-based HFNI valvometry. In a flow-through system, we simulated the contamination of running waters in the form of a short-term substance-peak, covering environmentally relevant as well as extreme concentrations of each substance.

The results indicate a substance-specific behavioral reaction of the mussels after short-term exposure, with immediate and delayed effects. These first results are based on state-of-the-art statistical comparison of simple measures such as count and duration of



opening/closing the shells. We currently advance the analysis by AI and machine learning-based methods to improve this promising new approach for early detection of potentially hazardous substances in the water.

## 1.12 The Use of 'Omic Tools to Assess the Impact of Mixture Toxicity

### 1.12.T-01 Linking Reproductive and Metabolic Disruption After Exposure to Novel Flame Retardants: An *in vitro* Approach

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Organophosphorus flame retardants (OPFRs) are widely used in several indoor and outdoor products as substitutes for polybrominated diphenyl ethers. The presence of OPFRs has been noted in various environmental matrices and biological samples, which indicates possible human exposure. The present study aims to evaluate the effects of OPFRs on human cell culture (monolayer and 3D spheroids) to characterize the toxicological effects and potential mechanisms through morphological, transcriptional, and biochemical assays. To evaluate the potential endocrine-related adverse reproductive effects (adrenal steroidogenesis), we further used the human adrenocortical cell culture (H295R).

Our findings suggest that the aromatic (aryl-) OPFRs, i.e., tricresyl phosphate (TMPP), triphenyl phosphate (TPHP), 2-ethylhexyl diphenyl phosphate (EHDPP), and the chlorinated OPFR, tris(1,3-dichloropropan-2-yl) phosphate (TDCIPP) induced the lipid accumulation in human liver cell culture by altering the expression of genes encoding for hepatic lipogenesis and mitochondrial dysfunction. Available data from ToxCast and *in silico* analysis suggested pregnane X receptor (PXR) and peroxisome proliferator-activated receptor-gamma (PPAR $\gamma$ ) as potential molecular initiating events. Moreover, EHDPP-mediated dysregulation of hepatic lipidome was also observed in human 3D hepatospheroids. Dysregulation in several lipid classes, including sterol lipids (cholesterol esters), sphingolipids (dihydroceramide, hexosylceramide, ceramide, sphingomyelin), glycerolipids (triglycerides), glycerophospholipids, and fatty acyls were noted along with alteration in several genes involved in lipid homeostasis. Potential effects on oxidative homeostasis were also noted. The H295R cells exposed to EHDPP showed altered production of hormones, including progesterone, androstenedione, and cortisol along with the enhanced expression of several corticosteroidogenic genes. Intracellular lipid assay identified EHDPP-mediated disruption of lipid profile in H295R cells as evidenced by reduced total cholesterol ester, sphingolipids, and increased phospholipids and triglycerides species, indicating connections between OPFR-induced metabolic and reproductive pathologies.

In summary, our study identified several OPFRs as potential risk factors for endocrine-related reproductive and metabolic pathologies that are of increasing importance due to the risk of occupational or cumulative environmental exposure to humans.

### 1.12.T-02 Using 'Omic' Data to Predict Chemical Sensitivity Across Terrestrial Invertebrates: Progress and Challenges

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Some terrestrial invertebrate groups are declining, with toxicant exposure thought to be an important driver. Using 'omic' data to predict differential species sensitivity (DSS) to toxicants, as well as vulnerabilities to chemical interactions, represents a cost-effective way to help flag species vulnerable to specific chemicals (and their combinations). However, this ambition requires uncovering the molecular mechanisms that underlie DSS, as well as determining the extent critical mechanistic differences between species can be extrapolated from readily available 'omic' data. We have used an approach that compares and scores genes/gene families pertinent to species sensitivity across three omic levels – (i) genomic (the presence/absence/functional domain sequence), (ii) basal gene expression (i.e., the level of expression in unexposed animals), and (iii) the responsive expression capacity (the extent and speed of omic response once exposed). 'Pertinent' in this case includes toxicant receptor genes (target and off-target), xeno-metabolic pathways, damage repair/mitigation systems and genes associated with genome responsive capacity (e.g., epigenetic regulator genes). Correlating such data with chemical sensitivity across terrestrial invertebrates identifies mechanistic insights into the basis of DSS, suggesting an under-appreciated role for off-target receptors, as well as confirming the role of xeno-metabolic complexity in determining a species' vulnerability to synergistic chemical interactions. This work is now expanding to correlating a species' 'responsome', i.e., the differential, and multi-dimensional, capacities of species to respond to toxicant-induced stress. Scoring a species' omic 'responsome' offers a rapid approach to quantify deep evolutionary divergences between species, as the score reflects differences in life-cycle, diet, reproductive strategy, regenerative ability, as well as a species' likelihood of experiencing unpredictable environmental extremes. Furthermore, given that many of the most problematic contaminants generate toxicity largely via non-specific modes of action, we suggest the 'responsome' represents a critical predictive factor that could augment current risk assessment. We hope that our approach and findings will support efforts towards combining omics with other appropriate data (e.g., ecological, phenotypic, and ecotoxicological) to identify vulnerable terrestrial invertebrates.

### 1.12.T-03 Effect of Wastewater Treatment Plant Discharge in a Norwegian Fjord to Juvenile Atlantic Cod (*Gadus morhua*) Brain Transcriptome

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The release of pharmaceuticals and personal care products (PPCPs) into the aquatic environment from runoff, aquaculture, and

effluent from wastewater treatment plants (WWTPs) may threaten the health of aquatic organisms. To better understand the input and movement of PPCPs from a predominant, regional WWTP into the Stavanger fjord and potential toxicity to an environmentally and economically important fish species, a dose related Risk and Effect Assessment Model (DREAM) was used to track the dispersion of complex chemical mixtures from the discharge plume. This model was used to select locations within the fjord to monitor the movement of the discharge and assess potential toxicity to Atlantic cod (*Gadus morhua*). Fish were caged at three sites for four weeks within the fjord. Brains were dissected from male and female individuals and transcriptomic profiling assessed, with differentially expressed genes presented relative to a reference location, site 1. The top predicted canonical pathways in male Atlantic cod caged at the site closest to the WWTP discharge, site 2, were involved in estrogen, glucocorticoid, and aryl hydrocarbon receptor signaling pathways, with disease and functions related to impaired growth of genital organs and neural cell proliferation, as determined through pathway analysis software. PPAR $\alpha$ /RXR $\alpha$  activation, neuroinflammation signaling pathway, and glucocorticoid receptor signaling were among the top predicted canonical pathways in male fish caged at the site furthest from the WWTP discharge, site 3, with disease and functions involved in the transport of molecules and central nervous system inflammation. Top predicted canonical pathways in female Atlantic cod caged at site 1 were involved in CREB signaling in neurons and axonal guidance signaling, with motor dysfunction/movement disorder and hyperactivity among the top disease and functions altered. Synaptogenesis signaling pathway and glucocorticoid receptor signaling were among the top predicted canonical pathways in female fish caged at site 2, with disease and functions involved in the formation of intermediate filaments and dissociation of neurofilaments. These data indicate that PPCPs in the proximity of the WWTP discharge are dispersed from the plume into rearing habitats for native fish species and compounds present in the discharge have multiple targets within the brain that impair motor neuron function and induce estrogenic effects.

#### 1.12.T-04 Earthworms Facing Pesticide Residues in Agricultural Landscapes

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Although agricultural soils are the main entry of pesticides, spatio-temporal and large-scale monitoring studies on soil and fauna contamination are still very rare, especially on currently used pesticides. There is consequently a lack of knowledge on the dynamics and fate of the pesticide contamination in agricultural landscapes. Research objectives of this study were threefold: 1) Characterize the multiresidual contamination of soils by pesticides and establish the spatio-temporal patterns of contamination according to agricultural soil management; 2) Analyze how earthworm's exposure is related to the soil pesticide contamination and 3) Assess how soil agricultural management differentially affects earthworms communities. Adopting a synchronic approach, 27 French agricultural fields were sampled (9 permanent meadows, 9 conventional fields and 9 organic fields), out of which 6 cropped fields were four time monitored through a diachronic approach. Both soil and earthworms were sampled and analysed. Multi-class pesticides (73 selected pesticides) and glyphosate and its metabolites have been determined concomitantly by LC-MS/MS. In soil, a total of 50 pesticides were detected in conventional fields, 35 in organic fields and 18 in meadows. Glyphosate and AMPA were found in all samples, including permanent meadows corroborating its ubiquity in the environment. Atrazine, Epoxiconazole and Fluxapyroxad were the most detected molecules apart from Glyphosate and AMPA. In earthworms, a total of 32 pesticides were detected from conventional fields, 16 in organic fields and 10 in meadows. High concentrations of Metolachlore ESA, Imidacloprid, AMPA and Glyphosate were found in earthworm tissues. Multivariate analyses will allow us to discriminate and assess the relative contribution of each agricultural and environmental variables (agronomical properties, farming practices, soil properties, inorganic pollution) in addition to the soil contamination by the pesticides that may explain the composition and structure of earthworm communities. This study showed that earthworms faced a cocktail of several chemicals at high concentrations in their natural environment. It thus raises important concerns about the effect of these residual pesticides on non-target soil organisms and how soil fauna may cope with that residual contamination in the future. Our observations advocate for more investigations on the evolutionary responses of soil biota to pesticide pollution.

#### 1.12.T-05 Effects of Mixtures of Herbicides on Soil Functions

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Despite the intensive and widespread use of pesticides, assessing environmental risks of mixtures on soils remains highly challenging. In this study, we used a mesocosm set-up to evaluate the effect of increasing doses of a commonly used herbicide scheme on biological nitrogen fixation and mycorrhization, and its relation with plant growth. This work aims to contribute to a more comprehensive environmental risk assessment of herbicides, by including more realistic test conditions and non-traditional endpoints.

The pot experiment was conducted inside a fine-meshed tunnel outdoors and lasted 100 days. The mixtures of herbicides were applied in two moments. On day 1 we applied glyphosate + dicamba + clethodim, and 30 days later, we applied S-metolachlor + flumioxazin. Four different concentrations were tested: 0 (control), 0.5, 1, and 2 times the recommended dose of the individual herbicides, referred to as D0, D0.5, D1, and D2 respectively. Each treatment had 10 replicates, but plants were harvested taking five random replicates in the vegetative stage (V4) and the remaining five plants in the reproductive stage (R3). Soybean seeds

inoculated with *Bradyrhizobium japonicum* were sown on day 35. On each time point, plant biomass, number, colour, and dry mass of nodules were registered. AMF were stained and the presence of hyphae, arbuscules, and vesicles was quantified.

In V4 stage, significant reduction effects were observed at higher herbicide doses on plant biomass, total mycorrhizal colonization and arbuscules, and nodules dry mass. In R3 stage treatments showed no significant differences in plant biomass and arbuscules colonisation. However, by the reproductive stage, D2 presented lower total colonisation than the control, and lower nodule dry mass than all the treatments. The number of nodules was not statistically different among treatments in V4, but by the R3 stage, D0.5 showed a higher number of nodules than D2. In V4 stage, vesicle formation showed significant differences and values were lower at D1 and D2 than at D0.5, but not different from the control. By R3, D2 was the only treatment that presented significantly less vesicle formation than the control.

Overall, the effects of mixtures of herbicides had a marked effect on soybean plant biomass and the symbiotic endpoints analysed in the first stages of the plant. The highest dose tested presented effects on the symbiotic process that could not be reverted by the reproductive stage.

## 1.12.P The Use of 'Omic Tools to Assess the Impact of Mixture Toxicity

### 1.12.P-Th068 Metabolomics to Study the Impact of Diazepam and Irbesartan on Glass Eels (*Anguilla Anguilla*) and the Differences in their Migratory Phenotypes

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Since glass eels (*Anguilla anguilla*) are continuously exposed to contamination throughout their migratory journey through the estuaries, to a certain extent the fall in the population of this endangered species may be due to this exposure, which is especially acute in estuaries under high urban pressure. Metabolomics was used in this study to assess the effects of the previously selected contaminants, diazepam, and irbesartan, on glass eels. An exposure experiment to diazepam, irbesartan, and their mixture was carried out over 7 days followed by 7 days of depuration phase. After exposure, glass eels were individually killed using a lethal bath of anaesthesia, and then an unbiased sample extraction method was used to extract separately the polar metabolome and the lipidome. The polar metabolome was submitted to targeted and non-targeted analysis, whereas for the lipidome only the nontargeted analysis was carried out. A combined strategy using partial least squares discriminant analysis and statistical analysis (ANOVA, *t*-test, and fold-change analysis) was used to identify the metabolites altered in the exposed groups with respect to the control group. The results of the polar metabolome analysis revealed that glass eels exposed to diazepam-irbesartan mixture were the most impacted ones, with altered levels for 11 metabolites, some of them belonging to the energetic metabolism, which was confirmed to be sensitive to these contaminants. Additionally, seven lipids were also found dysregulated after exposure to the mixture. In the same vein, the same metabolomics approach was also used to find the differences in the metabolic profile of the two behavioural phenotypes of glass eels (migrants and nonmigrants). Nonmigrant glass eels showed lower overall metabolic values than migrant glass eels, and the lipidome analysis also showed alterations in the levels of eight cholesterol esters. These two findings offer new research perspectives to explain this facultative migration of glass eels and confirm the suitability of metabolomics in attempting to explain the differences between migrant and nonmigrant glass eels.

### 1.12.P-Th069 Oxidative Markers in Pulmonary Cells Exposed to Different Fractions of PM<sub>2.5-0.3</sub> Collected from Urban, Traffic, and Industrial Sites in Northern France

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The aim of this work was to study the relationship between the chemical composition of PM<sub>2.5-0.3</sub> collected from different influences and their oxidative stress potency. Thus PM<sub>2.5-0.3</sub> were collected from three different sites (urban, road traffic, and industrial) in the North of France, and were then chemically characterized for major and minor chemical species. Four different fractions (PM<sub>2.5-0.3</sub>, organic extract, water soluble extract, and nonextractable matter) were prepared for each of the three samples. After pulmonary cells exposure to 24 µg/cm<sup>2</sup> of PM<sub>2.5-0.3</sub> or PM-equivalent extracts (i.e., organic extract, water soluble extract, and non-extractable matter), oxidative stress was studied by quantifying ROS accumulation in cells, oxidative damage to proteins (carbonylated proteins), cell membrane alteration (8-isoprostane) and DNA damages (8-OHdG). PM was capable of inducing severe ROS overproduction and caused damage to proteins at higher levels than other fractions. Higher cell membrane and DNA damages were found with PM and organic extracts from the urban site. Correlation analysis showed that ROS concentrations were correlated with carbonylated proteins, DNA damages and membrane alteration while principal component analysis (PCA) showed a correlation between the amount of ROS in cells and the 8-isoprostane and carbonylated proteins. The results of classification confirmed the ability of PM to induce the highest damage to proteins and cell membrane.

### 1.12.P-Th070 Identification of Adverse Outcome Pathway (AOP) Relevant to Diesel Particulate Matter (DPM) Using Transcriptomics and Comparative Toxicogenomics Database Integrative Approach

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Diesel Particulate Matter (DPM) is one of the major harmful mixtures and is well known to cause respiratory diseases such as lung cancer, pulmonary fibrosis, and asthma. However, due to the lack and limitations of mechanism-based research in mixture toxicity, it is challenging to establish a toxicity pathway. In this paper, we proposed a potential adverse outcome pathway (AOP) of DPM by integrating omics data with the Comparative Toxicogenomics Database (CTD) and AOP wiki. First, to discover Molecular Initiating Event, we screened the activity of transcription factors according to DPM exposure and confirmed that it induces activation of Aryl Hydrocarbon Receptor. In addition, differently expressed genes (DEGs) in time-dependent were discovered using transcriptomics data and 288 pathways were identified through Reactome analysis. In CTD analysis for discovering potential diseases through DEGs, 7 respiratory diseases were identified. In another, 32 respiratory diseases were found by searching CTD for diseases related to Particulate Matter (PM). Transcriptomics and CTD integrative approach revealed lung cancer and related 1210 CGPD tetramers. Through overlapping CGPD tetramers and toxicity pathways, 79 pathways and 146 phenotypes were identified, which were clustered into 11 key events in the AOP wiki. Putative AOP leading to lung cancer was then constructed. Among the 15 Key Event Relationships (KER) between these events, 10 were in the AOP Wiki and 5 were newly discovered. This study presented an approach in combination with CTD, AOP wiki, and omics in developing qualitative AOP as a case study of DPM. In addition, as a future study, we plan to establish a quantitative AOP using the dose-response relationship of omics and apply it to risk assessment.

#### 1.12.P-Th071 Particulate Pollution in Lebanon: *In-vitro* Evaluation of PM Organic Extractable Matter in Human pulmonary Cells

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Nowadays, air-pollution-derived fine particulate matter (PM<sub>2.5</sub>) is fully acknowledged to be a significant public health problem. PM<sub>2.5</sub> generally corresponds to a complex mixture of both inorganic (e.g., metals, ions) and organic (e.g., polycyclic aromatic hydrocarbons, pesticides, dioxins) chemical and biological components (e.g., pollen, fungi). The critical role of the PM<sub>2.5</sub> organic extractable matter (OEM) in the observed adverse health effects is still fragmented. The objective of this study was to investigate the toxicological effects of OEM prepared from PM<sub>2.5-0.3</sub> and quasi-ultrafine particles PM<sub>0.3</sub> collected in the south of Lebanon, on normal human bronchial epithelial cells (BEAS-2B).

The chemical characterization revealed the presence of a wide range of organic chemicals, notably PAHs, Nitrated-PAHs (N-PAHs), and Oxygenated-PAHs (O-PAHs), Dioxins and Furans. Exposure of BEAS-2B cells to OEM revealed decreases in ATP activities and increases in LDH levels, especially after exposure to the quasi-ultrafine particles OEM. In agreement with the high concentrations of PAH in OEM, CYP1A1 and CYP1B1 showed a high transcription level in cells supporting the activation of the AhR signaling pathway, especially after cells exposure to OEM<sub>0.3</sub>. Consequently, genotoxic effects occurred in BEAS-2B cells with cell survival events and cell cycle deregulation, as supported by alterations of the protein expression of pP53, total P21, pH2AX, total MDM-2, pCHK-1 pCHK-1 and 2. Given the original data reported in this preliminary study, future complementary works are also needed to better decipher the critical role of OEM<sub>0.3</sub> and the activation of processes involved in autophagy, mitophagy, and/or cell senescence.

#### 1.12.P-Th072 Use of Transcriptomic Points of Departure (tPODs) to Assess Toxicity of Oil in Early Life Stage Atlantic Cod

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Transcriptomics dose-response analysis (TDRA) is a promising approach for predicting the *in vivo* toxicity of environmental contaminants from transcriptomics data. TDRA has potential applications to the field of environmental risk assessment, but has seldom been used in the context of environmental mixtures. Here, we apply TDRA to early life stage Atlantic cod (*Gadus morhua*) exposed to two different types of oil; ultra low sulfur fuel oil (ULSFO) and cold lake blend diluted bitumen (CLB). Co-larvae (~200 degree days) were exposed to seawater control or water accommodated fractions (WAFs) made using variable loadings (0.01, 0.1, 1, 10, 25 g/L) of each oil for 24 h. Concentrations of polycyclic aromatic hydrocarbons (PAHs) and other organic chemicals were measured in water using analytical techniques. Whole larvae were flash-frozen in pools of five and preserved for RNASequencing. Quality control, trimming, and quantification were performed on RNAseq data using EcoOmicsAnalyst. Subsequent steps were performed on ExpressAnalyst which included filtration of low variance, low abundance, and unannotated genes, log2-counts per million (logCPM) normalization, and differential expression analysis using EdgeR. Differentially expressed genes (DEGs) were identified with an FDR adjusted *p*-value of 0.05 and log2-fold change (log2FC) of 1. Transcriptomic points of departure (tPODs) were calculated for each oil type using FastBMD. The ULSFO and CLB oils each contained a distinct complex mixture of PAHs that increased with oil loading. At equal oil loadings the ULSFO exposed cod was associated with greater mortality than the CLB exposed cod. Many genes were dysregulated by both oil types, but a greater number of DEGs were detected in ULSFO (1349) than CLB (530) -treated samples. Many DEGs were associated with aryl hydrocarbon receptor signalling. Gene level tPODs were calculated for 624 and 222 genes in the ULSFO and CLB samples, respectively. The tPOD mode for ULSFO and CLB were 0.034 and 0.18 g/L, respectively, suggesting a good

concordance between tPODs and apical outcomes. Overall, *in vivo* endpoints, chemical analysis, tPODs, and DEGs all suggested that the ULSFO oil was more acutely toxic to ELS fish. Ongoing analysis is investigating pathway level tPODs. This work helps to highlight the potential of TDRA for evaluating the toxicity of environmental mixtures.

#### 1.12.P-Th073 Chemicals in Plastic Food Packaging Activate Cell Surface Receptors

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Plastics have a wide variety of uses, including the packaging of foodstuff and beverages. As plastics contain complex chemical mixtures that can leach into such food and drink, plastic food packaging is, thus, a relevant source of human exposure to synthetic chemicals. GPCRs are interesting targets for plastic chemicals because they are the largest class of cell surface receptor and are central regulators in most cellular processes. Further, GPCR deregulation and dysfunction is linked to a multitude of diseases which is underscored by the fact that they are targeted by more than one third of all prescribed pharmaceuticals. In this work, we report activation across 132 GPCRs by bisphenol A (BPA), diethyl phthalate (DEP), triphenol phosphate (TPP), and three mixtures of the chemicals extracted from 14 plastic food contact materials. We demonstrate that plastic chemicals activate adenosine 1 (ADORA1), melatonin 1 (MTNR1A), melatonin 2 (MTNR1B), and melanocortin 3 (MC3R) receptors which have previously remained largely undescribed as targets for plastic chemicals. We further demonstrate that specific polyvinyl chloride and polyurethane plastic products contain MTNR1A and ADORA1 agonists. However, generalized concentration addition modelling indicates the presence of antagonism, synergism, and competitive antagonism within each mixture. We attempted to identify specific active chemicals within our mixture by docking all identified compounds against three crystal structures of MTNR1A and ADORA1. Finally, to understand the biological implications of our work, we performed a gene ontology analysis to identify commonality between MTNR1A, MTNR1B, MC3R, and ADORA1. The most prominent, shared biological processes function to align circadian and rhythmic behaviors, thereby indicating plastic chemicals may also act as circadian disrupting chemicals. Considering our continued and increasing production and usage of plastics, our results demonstrate the necessity of continued research to identify active chemicals, downstream effects, and their overall contribution to non – communicable diseases.

#### 1.12.P-Th074 Investigation of Benzotriazole Toxicity in Rainbow Trout Larvae (*Oncorhynchus mykiss*) using the Transcriptomics Point of Departure Approach

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Benzotriazole UV stabilizers (BUVS), which are a class of chemicals added to plastics as to increase their resistance to degradation by ultraviolet irradiation, are persistent and ubiquitous pollutants of aquatic strata. Detected at concentrations, *in situ*, ranging from a few nanograms per liter of water to several microgram per gram sediment and soil, even in pristine locations; BUVS are known to bioaccumulate and potentially bio-magnify in organisms and throughout the food web, respectively.

Toxicologically, BUVS can agonise and antagonise a wide array of biological functions, including a potential for endocrine disruption, impaired liver function and altered histopathology, activation of aryl-hydrocarbon receptor 2 mediated xenobiotic metabolism, as well as causing altered iron metabolism. The latter is believed to be related to increased oxidative stress.

In our ongoing study, we are aiming to assess the impact of two BUVS, UV327 and UV328, on early-life stages of rainbow trout (*Oncorhynchus mykiss*). Freshly fertilized eggs have been microinjected with serial doses of UV327 or UV328 and are being reared until complete yolk sac absorption. Upcoming termination of the assay, subsets of larvae will be used to assess a variety of apical endpoints (developmental malformations, histology) and for RNAseq. The objective is to identify apical and transcriptomic points-of-departure (aPOD and tPOD, respectively); that is, to identify doses at which the selected BUVS induce a shift in the whole-body tPOD and aPOD of exposed rainbow trout embryos. Subsequent data can then be utilized by environmental risk assessors to identify safe environmental concentrations, as demonstrated by several tPOD studies.

#### 1.12.P-Th075 Developing a Bioindicator Yoolbox for Monitoring the Tisk of Plant Protection Product Residues in Soil

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Soils contain complex networks of organisms which provide critical ecological functions allowing the balance, maintenance and resilience of the soil ecosystem. In agricultural soils, the functions performed by soil organisms play a critical role in maintaining soil fertility. However, agricultural soils can contain a range of plant protection product (PPP) residues, which can disrupt soil functions and soil fertility. To protect soil organisms and their functions, it is important to establish a toolbox of bioindicators to assess the impact of PPP residues on soil fertility.

The selection of a toolbox of bioindicators consisted of multiple steps. First, the actors (i.e., soil organisms) with a role in ecological soil functions supporting soil fertility were identified, based on key scientific references, using an ecosystem service framework to harmonize the information. Then, actors were ranked by their degree of connectiveness to different ecological processes and the relative importance of ecosystem services to soil fertility, as perceived by different stakeholders involved. The final scoring of the actors is the weighted (by stakeholder importance) connectiveness of organisms to processes and consequently ecological soil functions.

Bioindicator tools will be selected for the highest scoring actors, from available standardized guidelines or well-established test methods. The selection of the most appropriate methods will be performed in consultation with international experts for each key actor group, based on the applicability, cost and quality of data generated for each method. In addition to “ready to use” methods, experts will be inquired on promising methods that require future developments. The final toolbox selection aims to include both laboratory (ecotoxicological) and field (ecological) indicators.

In next steps, the bioindicator toolbox will be experimentally tested in pilot studies. Pilot studies aim at further refining the toolbox, establishing the sampling experimental design and developing normal operating ranges for bioindicators. Establishing normal operating ranges is critical to discriminate the effects of PPP residues from natural variability within a monitoring scheme.

#### **1.12.P-Th076 Proposal for the Validation Process of the Methodology for the Quantitative Analysis of Microorganisms**

*Josep Antoni Aguilar-Alberola, Carmen Gimeno and Adrian Martinez, Eurofins Trialcamp, Spain*

A large part of the studies for the validation process of the methodology for the quantitative analysis of microorganisms are based on the SANTE/2020/12830, Rev. 1 (2021). However, this guidance, as indicated by itself, is not intended for biological agents. Despite this, due to the lack of specific guidelines to carry out these studies, the methodology described in this poster is based on the principles indicated in SANTE, but adapted to the peculiarities of microbiology. The main reason for this is that the microbiological analysis is direct and does not depend on the signal of a detector as happens for chemical products.

The peculiarities associated with the storage of samples are also considered, since, in most cases, there are no methodologies described for the preservation of samples that contain microorganisms, whereas the stabilization and preservation methods for chemical products are known (in most cases by freezing) that allow the storage of samples for their later analysis.

A very important point to take into account in microbiology are the reproducibility conditions during the analysis of the samples, while aspects such as linearity and LOD are not really important. Furthermore, due to the intrinsic variability of microbiological methods, parameters such as the allowable recovery range, as well as specificity, must be reviewed and adapted. The matrix effect also deserves special mention, since, in microbiology, this effect may not simply be affecting its detection, but rather the viability of the microorganism.

#### **1.12.P-Th077 Evaluation of the Effects of a Sequential Application of Plant Protection Products on Soil Microbes and Free-Living Nematodes in a Field Experiment**

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Plant protection products are sprayed in conventional agriculture and integrated pest management to ensure an adequate production of food and fibers. During the growth cycle of a crop, several different plant protection products (PPPs) are usually applied, in combination or separately. Such sequential applications of PPPs result in a mixture of residues of active ingredients (a.i.) in the soil. The residues of a.i. are then dispersed in the environment and can reach nontarget organisms in the soil, including soil microbial communities, and may affect ecosystem services supported by these communities. This mixture scenario, rather frequent, has rarely been addressed experimentally. In this regard, the EFSA (European Food Safety Authority) recently published a scientific opinion (2017) inciting to include more soil microbe-mediated processes in environmental risk assessments of agrochemicals. In addition, PPPs could also affect free-living nematodes, a soil health indicator that is not well studied yet.

The objective of this study was therefore to evaluate the effect of the sequential application of PPPs on soil microbial community structure and functioning, and on free-living nematode abundance. To do so, triplicated plots in the field were treated either with an herbicidal PPP (150 g/ha clopyralid), an insecticidal PPP (75 g/ha cypermethrin), a fungicidal PPP (250 g/ha pyraclostrobin), or their sequential mixture at 1x or 10x the agronomical dose. Sequential applications were done one week apart from each other, and plots were sampled one day before the respective applications and day 7 and 28 after applications. Control and mixture plots were sampled at every timepoint over the 45 days. The ecotoxicological impact was evaluated on bacterial and fungal communities' diversity and structure using sequencing of 16S rDNA and ITS gene amplicons, and on the abundance of nematode.

We hypothesized that the mixture resulting from the sequential application of PPPs, especially at the highest dose applied, would have a stronger impact the structural and functional endpoints than the sequential application of PPPs. The results from this work will provide new knowledge and insights for the scientific community and may help fill the regulatory gaps on the assessment of risk of PPPs mixture for in soil living organisms.

#### **1.12.P-Th078 Differing Sensitivity of *Folsomia candida* and *Encytraeus crypticus* to Pesticides and their Mixtures**

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In agricultural environments soil organisms are often exposed to mixtures of pesticides. Understanding and assessing mixture exposures requires consideration of the potential interactive effects of chemicals. In combination chemicals may have differing

toxicity to what is expected if synergistic or antagonistic interactions occur. Here, two soil detritivores were considered in acute pesticide toxicity assays, first in single chemical exposures then in binary mixtures, to assess the mixture effects and any differences between species toxicity. Juvenile *Folsomia candida* and adult *Enchytraeus crypticus* were exposed to pesticides; Azoxystrobin, Chlorothalonil, Clothianidin, Cypermethrin, Imidacloprid and Prochloraz (and selected mixtures of these), with survival monitored through time. This allowed for toxicokinetic (TK) - toxicodynamic (TD) models, the General Unified Threshold model for Survival (GUTS), to be used to predict the effects of mixture exposures through time which were compared with the observed survival data. The two species showed differing sensitivity to the tested pesticides, in the single chemical exposures, with the highest sensitivity being to Imidacloprid for *F. candida* and Cypermethrin for *E. crypticus*. Mixture studies showed within a binary exposure both synergism and antagonism may occur, varying with dose and time. Therefore, chemical combinations cannot always simply be labelled as synergistic or antagonistic. The TK and TD traits of each species may contribute to the differing species sensitivity seen here. Our ongoing studies are considering these, TKs - through uptake rates measured via radiolabelled pesticide exposures, and TDs - by using molecular analysis of pesticide target receptors.

#### 1.12.P-Th079 Assessment of the Oxidative Potential of Fine Particles PM<sub>2.5</sub> According to their emission sources

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Fine particulate matter (PM<sub>2.5</sub>) present in the atmosphere are the subject of considerable attention from the scientific community and public authorities because of their proven impacts on climate and health. PM<sub>2.5</sub> form a complex mixture of chemical compounds that can differ according to emission sources. To implement effective air quality management policies, a better knowledge of the sources that contribute to the concentration of air pollutants as well as the assessment of their respective effects on health is necessary.

The objective of this study is to estimate the oxidative potential associated with the sources of fine particles that contribute to the ambient concentrations of PM<sub>2.5</sub> in the Dunkirk area, located in the North of France.

PM<sub>2.5</sub> were collected on four sites influenced by urban, road traffic, industrial and rural emissions. PM<sub>2.5</sub> from all four sites were chemically characterized, notably for the quantification of metal(oid)s, water-soluble ions, total carbon, and water-soluble organic compounds (Water-Soluble Organic Carbon (WSOC), Humic-Like Substances (HULIS), carboxylic acid, sugars, quinones as well as Polycyclic Aromatic Hydrocarbons (PAHs).

Oxidative potential has been evaluated by acellular tests, namely the ascorbic acid assay (OP-AA) and the dithiothreitol assay (OP-DTT). These methods were chosen because they are easy to use, inexpensive, have high repeatability, and are complementary because they are not sensitive to the same chemical species and particle sources.

#### 1.12.P-Th080 Differential Gene Expression of Freshwater Macroinvertebrates Exposed to Pesticide Mixtures: A Pilot Study in Agriculture Areas in Southern Sweden

**Pedro A. Inostroza**, Sameer Hassan, Henrik Aronsson and Thomas Backhaus, University of Gothenburg, Sweden

The expansion and intensification of agriculture activities and consequently the associated pesticide pollution remain one of the most important human stressors to aquatic ecosystems worldwide. Pesticides are designed to interact with specific pests' target sites and disrupt their biological pathways, which ultimately will lead to reduce their abundances. Unfortunately, pesticides end up in nearby water bodies forming pesticide mixtures and affecting, in the same manner, nontarget organisms' pathways reducing their ecological functions and/or eventually their biodiversity. Aquatic benthic invertebrate fauna represents one of the Biological Quality Elements, which is used to determine the Ecological status of surface water bodies according to the EU Water Framework Directive. They are therefore extensively used as biological indicators to assess water quality. Nevertheless, a thorough understanding of the adverse biological consequences of pesticide mixtures at the pathways level under real exposure conditions is still absent. There is not a standard approach defined to unveil such effects and most of the evidence available is based on single gene assessment. In this study, an RNA-based-sequencing (transcriptomic) approach was applied to shredder invertebrate *Gammarus pulex* populations to face the mentioned challenge. Nontargeted gene assessment provides evidence of expected chemical-target site interactions but also allows the identification of novel pathways under chemical stress. Our results suggest an overall significant downregulation of invertebrate genes exposed to pesticide mixtures in small streams. We did also rank differentially expressed genes in order to generate a comparative expression map between invertebrate population living in stressed streams and reference conditions (low human impacts). Finally, a pathways enrichment analysis allowed us to identify classes of genes being over-represented under pesticide mixture stress and link these responses to particular pesticide groups.

#### 1.12.P-Th081 Effect of Pesticide Residue Mixtures on *Lumbricus rubellus*: The SPRINT Approach

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The risk assessment of pesticides have been based on single pesticides, which is not reflective of the reality. In realistic scenarios, pesticides are found in the environment as mixtures, therefore risk assessment of pesticides should be based on mixtures. Moreover, toxicity tests used for risk assessment have been based on the use of one or few standardized species, e.g., *Eisenia fetida*, which may not represent ecological realism. It is in this knowledge that our study also assessed pesticide mixtures on a

native earthworm species, *Lumbricus rubellus*. For this study, 11 case study sites - CSS (10 European and 1 South American) representing all EU biogeographical zones and eight (n = 8) main cropping systems. From the pesticide residue analysis of each CSS, five pesticides were prioritized as mixtures specific for each CSS. The mixture toxicity of the five pesticides were then assessed in a dose-response experimental design at environmentally relevant concentrations - measured environmental concentrations (MEC), EFSA predicted environmental concentration (PEC) and five times PEC (5 x PEC) and control (no pesticides) for the eleven CSS. The toxicity of the pesticides varied across the CSS with some significant effect observed at levels below PEC in some CSS and some CSS did not show any effect of the pesticides at all concentrations tested. The results are relevant for risk assessment of pesticide mixtures in EU representative of cropping systems and biogeographical zones, using an important soil ecological receptor native to Europe.

#### **1.12.P-Th082 Compare the Effects of Different Mixture Pesticides on Earthworm**

**Fanrong Meng**, Wageningen University & Research, Netherlands

Increased use of pesticides has also resulted in the bio-accumulation and diffusion of pesticides and/or their metabolites in soil, water, and atmosphere which may eventually pose threats to environment and human health. Due to the ecosystem function of earthworms in the soil, they are often exposed to pesticides and are sensitive. Therefore, earthworms are selected as bioindicator species for contamination in the soil. Although most studies including the current regulations and legislations are mainly focused on single contaminants, it is apparent that the pesticides that occur in agricultural fields are mixtures instead of a single contaminant. Apart from additive effects, the interactions of agrichemicals can be synergistic, or antagonistic, which is largely unknown. This current study assessed pesticide residues of arable soils in 10 EU member states, which are the Netherlands, Czech Republic, France, Portugal, Switzerland, Slovenia, Croatia, Denmark, Italy, and Spain. Based on their detected frequency and concentration, different mixtures made up of five pesticides each were selected for assessing the toxic effects of pesticides at environmentally relevant concentrations on soil ecosystem. Toxicity tests with these pesticide mixtures, following standardized test guideline with earthworms (*Eisenia Fetida*), were conducted. We hope the results can fill some knowledge gap between current single pesticide test and complexity of the in-situ soil situation.

#### **1.12.P-Th084 Bottom-up Proteomics Analysis for Adduction of the Broad Spectrum Herbicide Atrazine to Mammalian Histone Proteins**

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Chemical pollution remains a poorly understood human-driven environmental change, and the impact is largely unknown for humans exposed to mixtures of over 350 000 chemicals registered for industrial use. Proteins are the direct mediators of cellular processes and (potential) targets of chemical contaminants. There is estimated to be 20 000 proteins in the human proteome. Proteins can covalently adduct at active amino acid residues with endogenous or exogenous chemicals including drugs, pesticides or their metabolites. Longer term research has demonstrated that these protein-adducts might lead to multiple health issues. Therefore, identification of xenobiotics adducted to key proteins and identification of the sites of adduction within the protein are important for a better understanding of events underlying diseases and chemically induced adverse reactions. Xenobiotics can react with multiple proteins at multiple sites and identification and characterization of adducted proteins are complicated by analytical challenges. Atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino]-1, 3, 5-triazine) and is commonly and mainly used for control of broadleaf and grassy weeds. Histones are the major proteinaceous component of chromatin in eukaryotic cells and an important part of the epigenome. However, to our knowledge, there has been no research on the formation of atrazine adducts with histone. In this study, a bottom-up proteomics analysis method was optimized and applied to identify histone adduction by atrazine *in vitro*. Whole histones of calf thymus or human histone H3.3 were incubated with atrazine. After solvent-based protein precipitation, the protein was digested by trypsin /Glu-C and the resulting peptides were analyzed by high-performance liquid chromatography–tandem high resolution mass spectrometry (UHPLC-MS/MS). The resulting tryptic/Glu-C peptide of DTNLCAIHAK from calf thymus or human H3.3 was identified with an accurate mass shift of +179.117 Da in atrazine incubated samples. A chemical group with elemental composition of C<sub>8</sub>H<sub>13</sub>N<sub>5</sub> (179.1171 Da) from atrazine adducted with calf thymus or human histone H3.3. MS/MS analysis confirmed that the adduction position was at its cysteine110 residue. Time and concentration dependent assays also confirmed the formation of histone H3.3 covalent adduct with atrazine *in vitro*. Thus, the potential exists that atrazine adduction may lead to the alteration of histones that subsequently disturbs their normal function.

#### **1.13 Towards a One Health Approach to Integrating Human Toxicology, Ecotoxicology and Ecological Impacts of PFAS and Related Chemicals**

##### **1.13.P-Th085 Lessons Learned: Considerations for PFAS Field Sampling Design**

**Sara J. Hutton**, Kenia Whitehead, Janet Anderson and Philip E Goodrum, GSI Environmental, Inc.

Per- and polyfluoroalkyl substances (PFAS) have emerged as contaminants of concern across an increasing number of sites undergoing remediation. The diversity of PFAS chemical characteristics combined with varying environmental factors has created a unique set of challenges in estimating and understanding how these compounds bioaccumulate in different systems. Literature values for chemical-specific bioaccumulation factors (BAFs) range by several orders of magnitude across different sites and taxa and between laboratory and field study conditions. We examine how abiotic factors can improve predictive models of fish tissue concentrations, and whether sampling designs can be optimized to better describe the key factors that explain variability. In addition, there is ongoing debate regarding the science supporting different regulatory approaches to PFAS mixtures, ranging from treating all PFAS as a single chemical class to evaluating specific chemical groups or individual compounds. We examine



possible limitations to the single class approach given the variability in PFAS uptake and toxicity. Field sampling occurred in freshwater streams and included analysis of sediment, surface water, aquatic vegetation, macroinvertebrates, and fish for various PFAS compounds. Fish data include various trout, perch, bluegill, and bass species. For our analysis for risk assessment, we explored the associations between abiotic conditions (i.e., pH, conductivity, temperature, hardness, and dissolved/total organic carbon) and BAFs. The results from our study indicate that PFAS uptake in fish is mediated via direct and indirect pathways from sediment and surface water to organisms. Our analyses highlight the complex pathways of PFAS from abiotic media into fish and indicates BAF/BSAF ratios alone may not be sufficient to predict fish tissue concentrations from surface water and sediment. Variability in data between field sites emphasizes the need to design sampling methods with the intent of capturing a wide net to account for variables influencing BAF/BSAFs. Examples of confounding variables include 1) differences in sampling whole body vs. fillet tissue and 2) abiotic factors that influence bioaccumulation, here we found conductivity to be important when predicting PFOS concentrations in fish at our site.

### 1.13.P-Th086 Exposure and Adverse Effects of PFAS in Resident Passerine Birds Breeding Nearby a Perfluoropolymer Plant

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Per- and polyfluoroalkyl substances (PFAS) encompass a large, heterogeneous group of chemicals of potential concern to human and environmental health. Among PFAS, in recent years the attention has been focused on emerging, short-chain compounds, which are expected to be less persistent, bioaccumulative and toxic, but more used and discharged in the environment, than their legacy, long-chain relatives. Whilst the information on the occurrence, the fate and the potential toxicity of some legacy PFAS is consolidated, there is a lack of knowledge for emerging PFAS. The present study aimed at monitoring the occurrence and adverse effects of legacy and emerging PFAS mixture nearby a perfluoropolymer plant (PFP) located in the Western sector of the Po River valley (Northwestern Italy). The presence of legacy and emerging PFAS was measured in the eggs of two resident species, the European starling (*Sturnus vulgaris*) and the Great tit (*Parus major*), breeding in nest-boxes located nearby the PFP. The adverse effects, in terms of the onset of an oxidative stress condition, were assessed on blood sample of nestlings of the same species. The concentrations of both legacy and emerging PFAS measured in the eggs from nest-boxes located nearby the PFP were higher compared to those from a control site. Accordingly, a modulation of antioxidant defenses and an increase in lipid peroxidation levels were noted in *S. vulgaris* nestlings born nearby the PFP compared to conspecifics from the control area. Our results showed that the exposure to environmental mixture of PFAS nearby a PFP can induce adverse effects on free-living organisms, suggesting the necessity of field studies to assess the risk of these emerging compounds.

### 1.13.P-Th087 Pre- and Postnatal Exposure to Per- and Polyfluoroalkyl Compounds (PFAS). Unravelling the Early Exposure to “the Forever Chemicals”.

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The presence of per- and polyfluoroalkyl substances (PFAS) in numerous types of samples represents a latent threat to human health and for the environment. In fact, these compounds are frequently found in human serum samples and this presence has been associated with various physical alterations and behavioral difficulties in children and teenagers. However, the association between health problems and the exposure to PFAS is often inconsistent, challenging the assessment of chronic exposure.

In this work, we want to evaluate the bioaccumulation of PFAS through the analysis of plasma samples (n = 498) in children of various ages (4, 8, and 12 years) and three origin cohorts from Spain (Gipuzkoa, Sabadell, and Valencia). Moreover, the links of a chronic exposure to these compounds by the drinking water supply will be assessed by analyzing actual occurrence of PFAS in those cohort areas as well as the different behavior impairments.

To this end, a targeted instrumental analysis method for the quantification of the largest possible number of PFAS in both plasma and water samples through High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometer (HPLC-HRMS) has been optimized. Moreover, different extraction protocols specific for each matrix have been tested to establish the most adequate procedure for each case, making a complete validation of the selected method.

In this sense, the presence of these compounds in plasma samples has been studied and the different statistical approaches will give us a new perspective about the early exposure profiles in plasma of children and teenagers. Also, drinking water analyses will give us information about PFAS levels at one of the most relevant exposure sources.

### 1.13.T-01 Macroinvertebrate Community Responses to PFAS Pollution: Identification of Threshold Body Burdens

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The pollution of perfluorinated alkyl substances (PFAS) in aquatic environments is a worldwide environmental concern with complex regulatory challenges. Today, most scientific studies focus on the distribution and bioaccumulation of PFAS while the ecological impact of PFAS contamination on aquatic communities is less studied. This lack of understanding goes along with a

lack of regulatory measures such as the derivation and implementation of suitable quality standards (QS). In Europe, biota and environmental quality standards have only been set for a limited amount of PFAS, while more than 4000 different compounds are produced. Moreover, the current biota quality standard (BQS) for PFOS was derived for the protection of human health and secondary poisoning of top predators. Therefore, the derived BQS might not be adequate for the protection of aquatic communities.

To study the impact of PFAS on a community level, we assessed the macroinvertebrate community structure from 35 rivers and streams across Flanders, Belgium. Aquatic macroinvertebrate communities can reflect the cumulative impact of short- and long-term pollution events because some taxa are more sensitive to pollution than others. At each site, accumulated PFAS concentrations were measured in the biotic (invertebrates) and abiotic environment (water and sediment). Additionally, physicochemical water and sediment properties such as pH, conductivity, cation exchange capacity, clay content, and total organic carbon (TOC) were assessed. The main aim of this research is to study the relationship between the accumulated PFAS levels and the invertebrate community structure to derive threshold body burdens that are protective of aquatic communities. To assess the community structure the multimetric index of Flanders (MMIF) was used. Secondly, we aim to evaluate the existing QS for biota, sediment and water by comparing them with the derived thresholds from this research. Lastly, we aim to study the relationship between PFAS in the biotic and abiotic environment and the influence of physicochemical properties on this relationship.

**1.13.T-02 Acute Toxicity of Seven Perfluoroalkyl Substances (PFAS) in *Daphnia magna* and *Raphidocelis subcapitata***  
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Among environmental pollutants of emerging concern, perfluoroalkyl substances (PFASs) are one of the classes of compounds most in the spotlight. Currently, there are few or as yet unknown effects that PFAS emissions may cause on aquatic ecosystems. Among the thousands of known PFASs, this research project evaluated the toxicity of several compounds that are normally found in the environment. Some of them are well-known compounds, such as PFOA and PFNA, while others are considered to be replacement compounds for new-generation PFASs, such as PFHpA, PFHxA, PFBA, PFBS, and GenX. As the Environmental Protection Agency is constantly seeking updated toxicological data to assess the toxicological framework of PFASs, this study provides toxicity data on two aquatic trophic levels. PFAS toxicity was evaluated on *Daphnia magna* according to the OECD 202 test and on *Raphidocelis subcapitata* according to the US-EPA OCSPP 850.4500: Algal Toxicity test. Among the two species, it was generally observed that algae are more sensitive to PFAS than daphnia. In fact, the EC<sub>50</sub> value for the compound under analysis was lower for algae (EC<sub>50</sub> mg/L: PFNA, 90.5; PFOA, 45.54; PFHpA, 385.98; PFHxA, 957.42; PFBA, 30.03; PFBS, 105.8; GenX, 738.0) than the Daphnids (EC<sub>50</sub> mg/L: PFNA, 187.5; PFOA, 705.9; PFHpA, >1000; PFHxA, >1000; PFBA, >1000; PFBS, >1000; GenX, 785.7). These data suggest that the alga *Raphidocelis subcapitata* is more sensitive to these molecules than *Daphnia magna*. As noted by previous research, PFASs can impair the photosynthetic system, causing downstream alteration of cell duplication and inhibition of algal growth. Since it appears that *Daphnia* is not the most sensitive organism to these molecules in acute exposure, this does not mean that these compounds cannot cause reproductive and growth damage in chronic exposure. In fact, it has been observed that following the organisms for another ten days after acute exposure the toxicity is more severe than that observed at 48 h of exposure, confirming the delayed toxicity. These data will be crucial to better understand the real impact of these molecules on the environment. In addition, they will be useful in laying the groundwork for further research on the chronic toxicity of PFASs and their possible interaction in mixtures.

**1.13.T-03 Per- and Polyfluoroalkyl substances (PFAS) Exposure Dysregulates Neural Activity, Exacerbates Microglial Injury Responses, and Sensitizes Larval Zebrafish to the Convulsant Pentylenetetrazole**

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Per- and polyfluoroalkyl substances (PFAS) are global contaminants that are classified as presumed immune hazards due to their adverse effects on adaptive immunity. However, comparatively little is known about how PFAS exposure affects the innate immune system. Microglia, the innate immune cells of the brain, are essential for responding to pathogens and injury, and also play critical roles in shaping brain development and homeostasis. Our prior work using zebrafish demonstrated developmental exposure to perfluorooctane sulfonate (PFOS) produces an activated microglial morphology and results in the upregulation of *p2ry12*, a G-coupled protein receptor involved in microglial activation and migration. Perfluorooctane sulfonate-induced microglial activation resulted in a heightened microglial response to brain injury, which could be rescued by using optogenetics to drive microglia toward a homeostatic state. We found the PFOS exposure increased neural activity and that the neuronal signaling environment modulated microglial activation state. Optogenetic silencing of the neurons was sufficient to normalize the microglial morphology and response to injury in PFOS exposed larvae. To further examine the relationship between immunotoxic PFAS congeners and neuron-microglial communication, we exposed zebrafish larvae to perfluorooctanoic acid (PFOA), an 8-carbon PFAS with a carboxylic functional group. Exposure to PFOA did not alter microglial responses to injury or the neuronal signaling environment, suggesting PFAS congeners have differential effects on neural activity. Consistent with this interpretation, we found perfluorohexane sulphonic acid (PFHxS), but not perfluorobutane sulfonic acid (PFBS) increased brain activity. Given that PFOS and PFHxS heightened neural activity, we next asked whether low level exposure to PFOS (2  $\mu$ M) or a mixture of PFAS sensitized zebrafish larvae to the GABAA receptor antagonist and convulsant, pentylenetetrazole (PTZ). Exposed larvae were incubated in subthreshold concentrations of PTZ that do not elicit seizures in control larvae. Behavioral assays revealed a differential response to PTZ in larvae exposed to the PFOS (2 $\mu$ M) or a PFAS mixture that contains equal proportions of PFOS,

PFHxS, PFBS, GenX, and PFOA at a concentration of 1  $\mu$ M/congener. We are currently performing electrophysiological recordings in PFAS-exposed larvae both with and without subthreshold concentration of PTZ to better understand the impact of PFAS exposure on neural systems.

#### 1.13.T-04 Neurotoxicity of Novel Perfluoroalkyl Ether Carboxylic Acids (PFECAs) in Earthworms (*Eisenia foetida*)

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New generation PFASs often incorporate one or more ether groups into their chemical structure for the purpose of reducing environmental persistence and potential bioaccumulation in biota. However very little is known about their toxicological properties and for this reason they are also called poor-data PFAS.

In the nematode PFAS (sensu strictu PFOS) neurotoxicity is mediated by mitochondrial impairment and superoxide stress. Using Perfluorooctanoic acid (PFOA) as a reference compound, we investigated the neurotoxic effects of three novel perfluoroalkyl ether carboxylic acids (PFECAs) with carbon chain between C4-C6 (PF3MOPrA, PF4MOBA, HFPO-DA) in the terrestrial oligochaetes *Eisenia foetida* exposed by means of the filter paper test (OECD Test.207).

Acetylcholinesterase (ACHE) activity was measured in earthworm tissues extract (SN10) obtained from animals exposed for 72 h to PFOA or each PFECA congener in a range of 0.6-229  $\mu$ M. PFOA elicited an overall increase of ACHE activity with some characteristics of hormesis suggesting potential anticholinergic effects. PF3MOPrA and PF4MOBA showed a similar trend, while a moderate threshold inhibition was observed for HFPO-DA.

SOD activity was also evaluated by means of in gel assay. Preliminary results seem to indicate a dose dependent increase of enzyme activity in tissue protein extracts of PFOA and PFECAs.

To confirm the development of neurotoxicity triggered by PFAS neurotransmitter measurement is ongoing by means of a targeted LC-MS approach in a UHPLC Vanquish system coupled with an Orbitrap Q-Exactive Plus, identifying and quantifying more than forty molecules and precursors such as GABA, melatonin, serotonin, TMAO, acetylcholine, tryptophan, indols, dopamine. A set of behavioural tests will complete the neurotoxicological assessment to classify the hazard of next generation PFAS.

#### 1.13.P Towards a One Health Approach to Integrating Human Toxicology, Ecotoxicology and Ecological Impacts of PFAS and Related Chemicals

##### 1.13.P-Th089 Towards an Integrated Approach for Testing and Assessment of PFAS – Integration of Experimental and Computational Approaches, from Literature and Developed within SCENARIOS

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Per- and polyfluorinated alkyl substances (PFASs) are a large, complex group of manufactured chemicals with widespread use in aerospace, automotive, construction, and electronics industries. The OECD estimated that >4000 PFASs have been produced and used in a broad range of industrial and consumer applications. However, little is known about the potential hazards (e.g., bioactivity, bioaccumulation, and toxicity) of most PFASs, and the vast majority of data on the health effects of PFAS family chemicals have been done on just a handful of chemicals. Thus, for the vast majority of PFAS compounds there is insufficient data on hazard (or exposure) on which to make a risk assessment as to their safety for use in consumer products.

SCENARIOS is developing a computational approach to expand and extend the long-term toxicity assessment and risk assessment methods that have been applied for the well-studied PFAS members to members that are currently non-regulated PFAS, or for which there is very limited experimental data. The initially developed workflow contains modelling and data analysis components that can be adjusted to the available data in each case ('omics, *in vitro*, or *in vivo*), and is formulated as an Integrated Approaches to Testing and Assessment (IATA) for risk assessment of PFAS. In due course the approach will also be applied to PFAS congener mixtures, enhancing and enabling a one health approach to environmental and human health protection.

Here we present the first iteration of the IATA, which has been applied to a set of 34 data-poor PFAS molecules as the basis for the extension to the models to data-poor PFAS candidates, based on the k Nearest neighbour (kNN) approach. KNN is an instance-based (lazy) method that predicts the dependent variable based on the distance of the  $k$  ( $k = 1, 2, 3, \dots$ ) nearest neighbours. Based on binding data to transthyretin (TTR) a key membrane transport protein, cytochrome P450 1a, a protein involved in the biotransformation of chemicals in the liver, and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) the major component of the lipid bilayer, a set of QSAR models based on protein / lipid binding, bioavailability, and toxicity was established, and the experimental validation of the predictive power of the models was confirmed experimentally. The integration of the various models, supplemented by machine learning models from the literature, into a first version of an overall IATA for risk assessment of PFAS will be presented.

**1.13.P-Th090 Toxicological Assessment of PFAS in Embryonic Zebrafish Cells and its Implications for Human Health**  
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Per- and polyfluoroalkyl substances (PFAS) persist in the environment, bioaccumulate through the food web, and are toxic for humans. This class of chemicals is very broad and only a few members are well studied, leading to gaps in knowledge and limited risk assessment. To overcome this, alternative test systems are needed to enable screening of a wider range of PFAS chemicals and extrapolation of knowledge from data-rich PFAS to data-poor ones. Adverse Outcome Pathways (AOPs) are an emerging approach to organise molecular, cellular, and whole organism toxicity information in order to link from an initial exposure and molecular initiating event through a set of key events to an adverse outcome. Thus, other chemicals that trigger similar key events are likely to lead to the same adverse outcome, reducing the need for animal testing.

Here, we present results from a literature screening of PFAS toxicity and its organisation into an AOP, and building on this, toxicity screening data on a suite of 34 PFAS chemicals utilising a zebrafish embryonic cell model (ZF4), which is a suitable model to explore chemical toxicity in the aquatic environment and for extrapolation of effects in human beings. The suite of endpoints assessed includes cell viability, membrane permeability, oxidative stress induction, upregulation of multidrug resistance protein 4 and multiresistance-associated protein 1, and expression of glutathione S-transferase and cytochrome P450 1A associated with PFAS biotransformation. ZF4 cells were cultured in a DMEM/F12 supplemented with 1% penicillin and streptomycin at 28 °C and saturated with 5% CO<sub>2</sub> in a humidified atmosphere. Cells were exposed to three different environmental concentrations of each PFAS with three replicates. Dose-response curves were established, and modes of action and biotransformation assessed.

Based on the screening results, 10 PFAS will be selected for more detailed mechanistic studies in zebrafish, which will be exposed in semistatic conditions at environmental PFAS concentrations and sampled at regular intervals. For each sampling, several organs will be inspected for abnormalities and PFAS content will be evaluated via biodistribution studies and used to establish a zebrafish PBPK model for environmental risk assessment providing a maximum residue level which will help the authorities in determining the global regulatory PFAS levels.

**1.13.P-Th091 Perfluorooctanesulfonic Acid Induced Seizurogenic Effect in Zebrafish Larvae**

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While the developmental neurotoxicity of perfluorooctane sulfonate (PFOS) has been reported, its seizurogenic potential has not been investigated. Behavior assessment was conducted in zebrafish larvae exposed to PFOS at concentrations of 0, 0.1, 1, 5, 10, and 20 µM. Changes in electrophysiological signals and in the concentration of 20 neurochemicals were measured. Behavior assessment revealed that PFOS altered larval behaviors and significantly increased the counts and duration of bursting (an irregular high-speed movement). Electrophysiological analysis showed that the number of seizure-like events and duration of seizure-like signals were significantly increased, corresponding to results observed using pentylenetetrazol as a positive seizurogenic agent. The outbreak of seizures detected via abnormal electrophysiological signals was confirmed by the increased expression of c-fos and bdnf, which are typical seizure-related genes. Analysis of neurochemicals indicated that PFOS dysregulated overall neurotransmission systems, and aberrant endogenous concentrations of various neurochemicals in the amino acid, cholinergic, dopaminergic, serotonergic and kynurenergic, and GABAergic systems were associated with seizure-like behavior and signals. This study, the first to demonstrate that exposure to PFOS provokes a seizurogenic effect in developing zebrafish larvae, should stimulate further research on the association between PFOS exposure and neurodevelopmental toxicity or neurological disorders.

**1.13.P-Th092 Integration of Multi-Omics Reveals the Analogous Developmental Neurotoxicity Mechanisms Between PFBS and PFOS in Zebrafish**

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The differential biomolecular mechanisms between legacy per- and polyfluoroalkyl substances (PFAS) and their short-chained alternatives were studied at the same concentrations, which hampers our understanding of the potency of alternatives. We therefore compared the commonalities of PFBS and PFOS with profiled biomolecules of zebrafish larvae such as transcripts, proteins, and metabolites. PFBS showed approximately 700 times lower developmental toxicity than PFOS based on the benchmark dose (BMD) of embryonic mortality. Although the toxic potency was different, larval neurobehavior patterns were similar; hyperactivity was observed at the same ratio of lower concentrations on a BMD<sub>5</sub>, and constant larval movement was observed at higher concentrations. Biomolecular profiles from individual omics analysis also indicated the similarities between PFBS and PFOS in oxidative stress, immune response, lipid metabolism, and energy metabolism. Knowledge-based integration of multi-omics was further applied to better understand the neurotoxicity of PFBS and PFOS. The integrated multi-omics analysis revealed the commonality in enriched pathways affected by both PFBS and PFOS such as dysregulation of various signaling pathways, lipids metabolism, amino acid metabolism, and oxidative response. In particular, the biomolecular mechanism that underlies the similar neurotoxicity of PFOS and PFBS was closely interlinked, including calcium signaling pathway, primary bile acid biosynthesis, and peroxisome. These findings demonstrated that integrated multi-omics is useful to explore neurotoxicity of PFAS alternatives as well as PFBS could be an alternative of PFOS because of low neurotoxicity in developing zebrafish embryos. However, the similarly dysregulated biomolecular mechanisms suggested that the potential risk of PFBS should also be carefully managed.

### 1.13.P-Th093 Identifying Bioactive Per- and Polyfluoroalkyl Substances (PFAS) from a Diverse Library and Their Mode of Action in Zebrafish

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Per- and polyfluoroalkyl substances (PFAS) are a structurally diverse class of chemicals of particular interest due to their impacts on both environmental and human health. Toxicological data are only available for a small number of the thousands of PFAS on the global market, and even fewer PFAS with transcriptomic information. A more complete understanding of PFAS hazard potential and modes of action by which PFAS induce toxicity is needed. We assessed the developmental toxicity of a structurally diverse PFAS library in zebrafish and conducted a transcriptomic investigation of bioactive compounds to fill this data gap. We screened two libraries: 139 PFAS stocks procured from the US EPA and 25 PFAS obtained directly from manufacturers (144 unique structures). Embryonic zebrafish were statically exposed in 96-well plates to 0.015-100  $\mu$ M PFAS from 6 to 120 h postfertilization (hpf), and 10 morphological endpoints were evaluated ( $n = 36$ ). Fifteen PFAS, which included those with sulfonic acid, sulfonamide, diacrylate, and diol substituents, induced morphological effect(s) in at least 80% of the test population and were selected for subsequent transcriptomic interrogation. Exposures were conducted at the concentrations that induced 80% effect ( $EC_{80}$ ) by 120 hpf for each PFAS, sampling at 48 hpf ( $n = 4$  pools of 10 embryos), before observable effects. We utilized Lexogen QuantSeq 3' FWD mRNA-Seq library preparation and performed NextSeq mRNA sequencing which found differential expressed gene (DEG) counts from 0 to 3,715. Internal concentrations of PFAS were then measured to determine if differing uptake resolved the disparate DEG counts across the compounds. We identified high internal concentrations of PFAS at 120 hpf which indicated the possibility of differentiated uptake at other developmental stages and/or that the availability of biological targets occurs later in development. Finally, we measured gene expression changes across different life stages between 72-120 hpf for the chemicals that produced few DEGs at 48 hpf despite their production of robust 120 hpf phenotypes. Collectively, this study identified a subset of bioactive PFAS and their resulting gene expression perturbations across diverse chemical structures in a single *in vivo* system. These findings begin to elucidate the differential actions of individual PFAS in biological systems for improved regulatory decision-making.

### 1.13.P-Th094 Effects of PFAS Exposure on Telomere Length in Resident Passerine Birds Breeding Nearby a Perfluoropolymer Plant

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Telomeres are noncoding DNA repeats located at the end of eukaryotic chromosomes that play a key role in ensuring genomic stability. Although telomere length progressively shortens over individual lifespan with advancing age, the rate of telomere shortening can accelerate as a consequence of diverse environmental stressors. Among these, the exposure to environmental contaminants can contribute to the modulation of telomere length or dynamics. The present study aimed at investigating the effects due to the exposure to legacy and emerging per- and polyfluoroalkyl substances (PFAS) on telomere length of two bird species, the European starling (*Sturnus vulgaris*) and the Great tit (*Parus major*), living nearby a perfluoropolymer plant (PFP). In 2022, nest boxes were placed nearby a PFP located in the Western sector of the Po River valley (Northwestern Italy), as well as in control area. The first laid egg from each nest box was collected to estimate the maternally transferred amount of PFAS. A blood sample was collected from all the nestlings growing in the nest boxes and telomere length was measured through a real time PCR method. Previous correlative studies returned contrasting results on the relationships between the concentrations of PFAS and the telomere length in adults and nestlings of bird species sampled in remote areas. Thus, the preliminary results of the present study, performed nearby a PFP with high levels of PFAS contamination, allow to shed light on the relationships between the levels of both legacy and emerging PFAS, and telomere length in free-living birds.

### 1.13.P-Th095 Impact of PFAS on Lipid Accumulation and Cholesterol Homeostasis in the Human Hepatocarcinoma Cell Line HepaRG

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Per- and polyfluoroalkyl substances (PFAS) are a large family of synthetic chemicals that are used for numerous consumer products with water- and dirt-repellent properties. Due to their extraordinary stability they are resistant to thermal, chemical, and biological degradation and, therefore, persist in the environment. Some PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are toxicologically well characterized. Exposure to PFAS has been associated with several adverse health effects in humans such as increased serum cholesterol levels. As PFOA and PFOS are continuously replaced by alternative PFAS in the industrial applications, this study aims to characterize the impact of these PFOA and PFOS replacements on lipid accumulation and cholesterol homeostasis in human liver cells.

The human hepatocarcinoma cell line HepaRG was selected as *in vitro* model for human hepatocytes. In addition to PFOA and PFOS, we selected perfluorohexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS), perfluorobutanoic acid (PFBA), perfluorobutanesulfonic acid (PFBS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and two next generation PFOA replacements (GenX and ADONA) for *in vitro* testing. The steatotic potential of the 10 selected PFAS in HepaRG cells was examined using the AdipoRed assay to determine intracellular triglyceride accumulation. To examine the cholestatic potential of PFAS, we employed an *in vitro* assay that is based on the observation that in the presence of high bile acid

concentrations cholestatic compounds are cytotoxic already at lower concentrations compared to their cytotoxicity in the absence of bile acids.

In the AdipoRed assay, the selected PFAS induced no significant increase in triglyceride accumulation except for a slight increase for some PFAS at the respective highest noncytotoxic test concentration that was statistically significant in some cases. Regarding the experiments on the cholestatic potential, two well-known cholestatic drugs (cyclosporine A and nefazodone) clearly showed increased cytotoxicity in the presence of high bile acid concentrations. In contrast, cytotoxicity induced by the selected PFAS was independent of the presence or absence of bile acids. From our *in vitro* data we conclude that the selected PFAS neither induce triglyceride accumulation nor possess a cholestatic potential in HepaRG cells at concentrations that are relevant for human exposure scenarios.

### 1.13.P-Th096 Toxicity Mechanisms of Legacy and Novel Sulphonated Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are highly complex synthetic compounds used in several products and industrial processes (fluoropolymers, semiconductors, textile, chrome plating, chloro alkali, etc.). PFAS are mobile, omnipresent in the environment and extremely persistent. Although the toxicological implications of human exposure to certain (legacy) PFAS are well-studied, data on alternative/next generation PFAS are still very limited. Thus, collecting experimental toxicity information of such poor-data PFAS is of high importance.

The high serum-binding of PFAS congeners may influence their cytotoxic activity. Therefore, the influence of foetal bovine serum (FBS) and bovine serum albumin (BSA) on the cytotoxic activity of one legacy (Perfluorooctanesulfonic acid – PFOS, CAS number 2795-39-3) and seven poor-data PFAS belonging to the sulphonic acid series (CAS numbers: 3871-99-6; 763051-92-9; 29311-67-9; 2706-91-4; 375-92-8; 70755-50-9; 749836-20-2; 113507-82-7) was investigated. Two epithelial barrier *in vitro* models, i.e., A549 cells and a differentiated co-culture of Caco-2 HTB-37 and HT-29 MTX cells was exposed to the compounds in the presence of zero, low (1%) and high (10%) FBS, or BSA (1 mg/mL). Cells exposed to PFOS in the presence of BSA displayed an increased susceptibility to cytotoxicity than cells exposed in the presence of any or no level of FBS. This was observed for some, but not all, alternative PFAS. In contrast, cells exposed to 8:2 Cl-PFESA with no or low FBS tolerated a lesser concentration than cells exposed in high FBS or BSA. Also, a large discrepancy in sensitivity between the cellular models was observed, with the intestinal epithelium being the less sensitive system. This could in part be attributed to the models' inherent properties, yet it warrants further investigations into the specific toxicity mechanisms in different organ systems.

Future studies encompass the use of advanced *in vitro* 3D models (intestinal barrier, alveolar barrier, and skin) and single-cell transcriptomics. The results will inform on potential apical effects that can be measured in high throughput assays, and so contributing to a testing toolbox to improve and address the hazard of PFAS exposure to human health and ultimately, facilitate read-across actions between legacy and poor-data congeners.

### 1.13.P-Th097 Investigation of PFASs Interactions with Biomolecular Model Systems Powered by the Enalos Asclepios KNIME Nodes

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Per- and polyfluoroalkyl Substances (PFASs) are present in a wide variety of industrial processes and consumer products, the majority of which having unknown hazardous potential in terms of bioactivity, bioaccumulation, and toxicity. Understanding of their physical and chemical properties and how these determine their biological and toxicological effects is of great importance and will enable the prediction of their interaction properties, without the necessity of performing extensive empirical testing (and thus with a reduction of costs and need for *in vivo* testing). In this respect, the design of a comprehensive *in silico* study for the elucidation of the interaction mechanisms of PFAS molecules with biomolecular systems is necessary, especially those for PFAS for which limited experimental data is currently available. To this end, a plasma transport protein and a cellular membrane model are utilized, i.e., transthyretin (TTR) and the 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipid bilayer, respectively, as interaction targets. TTR is a transport protein present in human, mammalian, and fish plasma that transports the thyroid hormone thyroxine (T4) and retinol to the liver. TTR has a dimer of dimers quaternary structure (55kDa MW, 508 residues, ~4,065 atoms). DOPC membrane consists of lipids, each of which has a hydrophilic head oriented to the exterior and two hydrophobic tails oriented to the interior of the bilayer. The ability of PFASs to interact with biomolecules such as TTR and lipid bilayers is of great importance, since these molecules enable PFAS transport within the body. Understanding PFASs interactions with biomolecular systems may enable minimisation of their toxicity by designing alternative compounds that interact less and are thus less bioavailable. Our methodological approach involves using the Enalos Asclepios KNIME pipeline to perform molecular docking

and fully atomistic molecular dynamics (MD) simulations on a panel of preselected PFAS molecules, some of which have existing experimental data and some that are data-poor, for which the predictions will then be validated experimentally to confirm their interactions. The findings of this project are expected to assist toward understanding the interactions and transport mechanisms of PFASs in living organisms, thus allowing rational design modifications to minimise their environmental and toxicological impact and design alternative molecules with improved features.

### **1.13.P-Th098 *In vitro* Examination of Molecular Toxicity Mechanisms of Alternative (Poly-)Ether Per- and Polyfluoroalkyl Substances**

**Wiebke Alker, Thorsten Buhrke and Albert Braeuning, German Federal Institute for Risk Assessment (BfR), Berlin, Germany**

The use of many legacy per- and polyfluoroalkyl substances (PFAS) is being reduced due to their high persistence and hazardous effects. Thus, alternative PFAS are increasingly used for numerous industrial applications, while at the same time there is no or only few data on their toxicity.

The project aims at examining the molecular toxicity mechanisms of alternative PFAS with a focus on the subgroup of mono- and polyether PFAS, with both linear and branched structures. A number of perfluoroalkyl sulfonic acids (PFSA) and perfluoroalkyl carboxylic acids (PFCA) including the well-characterized perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were also included in the list of in total 34 different PFAS compounds that were selected for *in vitro* testing in the present study. The human cell line HepaRG was chosen as a model to study potential hepatotoxic effects of PFAS *in vitro*. Here we present data on the cytotoxic effects of the 34 PFAS in HepaRG cells and on the activation of the peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ), whose activation has been attributed to hepatotoxic effects of PFOS and PFOA in rodents.

Most monoether PFAS were less cytotoxic to HepaRG cells than PFOA and PFOS, whereas some linear and branched polyether PFAS displayed a higher cytotoxicity than the legacy PFAS. For most of the selected PFAS, PPAR $\alpha$  activation was only observed at concentrations close to the cytotoxicity level of the respective compound. However, some PFAS (e.g., 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (HFPO-DA) and perfluoro-4-methoxybutanoic acid) activated PPAR $\alpha$  already at concentrations much lower than their cytotoxicity levels, indicating that some PFAS may induce stronger effects at this particular receptor than PFOA or PFOS, although being less cytotoxic.

The *in vitro* data can be used to calculate benchmark concentrations and derive points of departure for specific cellular and molecular endpoints. A comparison of these data with those of the well-characterized PFOA and PFOS will support risk characterization of the poor-data PFAS examined in the present study.

### **1.13.V Towards a One Health Approach to Integrating Human Toxicology, Ecotoxicology and Ecological Impacts of PFAS and Related Chemicals**

#### **1.13.V-01 Developing an Easily Accessible Repository of PBK Models for PFAS**

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Physiologically based kinetic (PBK) models are a key component in both human and environmental risk assessment workflows. Used in a forward dosimetry mode, they can convert external exposure into internal organ burdens, while reverse dosimetry can estimate the external exposure that leads to a witnessed concentration at the tissue level. PBK models for per- and polyfluoroalkyl substances (PFAS) are invaluable tools for investigating the persistence and impact of this class of chemical substances in humans and other species. Ease of access and use of PBK models for PFAS can potentially accelerate the process of risk characterisation. We have developed a framework based on R (Python under development) for uploading and exposing PBK models as fully documented, ready-to-use web services through the Jaqpot platform, thus making them also available through the SCENARIOS cloud platform. Using this framework, we have replicated and exposed several PBK models for PFAS that have been published in literature, so that they can be used by nontechnical end-users. Clients in various programming languages (R, Python, Java, etc.) allow remote use of the models and easy integration in computational pipelines. Our goal is to continuously update the existing list of PFAS-related PBK models on Jaqpot, as long as new models emerge, so that these models can reach a wider audience, increasing their applicability.

#### **1.13.V-02 Systematic Developmental Toxicity Assessment of a Structurally Diverse Library of 139 PFAS in Zebrafish**

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Per- and polyfluoroalkyl substances (PFAS) are widely used chemicals with very limited human health data relative to the diversity of structures manufactured. To help fill this data gap, an extensive *in vivo* developmental toxicity screen was performed for 139 PFAS provided by the US EPA. Dechorionated embryonic zebrafish were exposed to 10 nominal water concentrations of PFAS (0.015 - 100  $\mu$ M) from 6-120 h postfertilization (hpf). The embryos were assayed for embryonic photomotor response (EPR), larval photomotor response (LPR), and 13 morphological endpoints. A total of 49 PFAS (35%) were bioactive in one or more assays (11 altered EPR, 25 altered LPR, and 31 altered morphology). Perfluorooctanesulfonamide (FOSA) was the only structure that was bioactive in all 3 assays, while Perfluorodecanoic acid (PFDA) was the most potent teratogen. Low PFAS volatility was associated with developmental toxicity ( $p < 0.01$ ), but no association was detected between bioactivity and five other physicochemical parameters. The bioactive PFAS were enriched for 6 supergroup chemotypes. The 14 most bioactive PFAS plus

the previously untested Nafion byproduct 2, an emerging next gen PFAS of concern, were selected for transcriptomic investigation. We sought to phenotypically anchor the transcriptional changes, thus the EC<sub>80</sub> for each PFAS was used as the exposure concentration and embryos were sampled at 48 hpf. GO analysis identified both common and unique biological processes enriched by structurally diverse PFAS including protein folding, regulation of peptidase activity, disrupted peptide biosynthetic and metabolic processes, translation, neuron development and differentiation, and sensory system and visual system development. Many of the most significantly enriched processes were related to nervous system, brain, and neuron development. The results illustrate the power of a multidimensional *in vivo* platform to assess developmental toxicity of diverse PFAS and to accelerate PFAS safety research.

### 1.13.V-03 Cytotoxicity of Ether Perfluoro Carboxylic Acid PFAS Congeners in Earthworm Granulocytes

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Soil pollution has enormously increased in the last decades. From different type of pollutants per- and polyfluoroalkyl substances (PFAS) are found in the soil due to their various industrial uses (e.g., anti-fire foams, fluoropolymer resins such as Teflon; separation processes; textiles; cosmetics) and their high persistence. Most emerging PFAS congeners do not have toxicity data that would allow an environmental assessment. Biological approaches to soil monitoring, such as the measurement of biochemical and cellular responses to pollutants (i.e., biomarkers) on organisms living in the soil, have become of major importance for the assessment of the quality of soil. The aim of this work was to investigate the effect of these substances from an ecotoxicological point of view on nontarget species, using PFAS as a reference standard. For this purpose, our study focused on assessing the potential cytotoxicity and genotoxicity of four different PFAS congeners (PFOA, HFPO-DA, PF4MOBA, PF3MOPrA) on immune system cells (coelomocytes) of sexually mature earthworm *Eisenia foetida* in a range concentration of 0.6-229 µM. Toxicity tests were carried out according to OECD Test No 207 (by filter paper test) to assess the cytotoxic and genotoxic effect in earthworms coelomocytes. Coelomocytes were collected from the coelomatic fluid using an insulin syringe prefilled with 0.25 ml saline solution. Coelomocytes morphometric alterations were determined by Diff quick staining; oxidative stress alterations and micronuclei frequency were determined respectively by H2DCFDA staining for ROS and DAPI coupled with fluorescence microscopy. Results showed significant alteration of the investigated patterns. An increased enlargement of granulocytes was usually observed in exposed earthworms with respect to control group. A hormetic pattern was observed. The enlargement was quantified by measuring the area of 2D digitalised granulocyte images. A decrease of oxidative burst and an increase of micronuclei frequency were also seen. Further investigation of impaired cell-mediated immune function is ongoing. The results of this study will lead to the construction of an ecotoxicological database on numerous alternative PFAS congeners allowing a weight of evidence risk assessment analysis of these substances. These data can contribute to regulation and restriction of PFAS at national and European level.

### 1.14 Using New Approach Methods to Move from Descriptive to Mechanistic Ecotoxicology

#### 1.14.P-We025 Targeted Phosphoproteomics Reveals the mTOR Pathway Dynamics and its Role in Growth Regulation in the Zebrafish PAC2 Cells

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Environmental assessment of chemicals frequently uses fish growth as a chronic toxicity endpoint, and alternative methods to replace this animal test are urgently needed. Previously, it was demonstrated that measurement of proliferation of fish cells cultured *in vitro*, in conjunction with toxicokinetic modelling, could be used to predict impacts of pesticides on fish growth *in vivo*. However, the molecular mechanisms underlying chemical effects on fish cells growth remain poorly understood. We here focus on the mechanistic target of rapamycin (mTOR) pathway, which is known to regulate cell growth and proliferation in mammals. Since the mTOR pathway activity is regulated by protein phosphorylation, it has been traditionally studied by antibody-based assays. However, suitable antibodies are often lacking for nonmammalian species. Moreover, antibody-based methods do not allow monitoring multiple pathway targets simultaneously. To overcome this challenge, we developed an alternative approach using mass spectrometry-based targeted (phospho-)proteomics, first applied in the zebrafish (*Danio rerio*) PAC2 cell line model. Our method currently allows monitoring changes in protein abundance and phosphorylation of 15 protein targets along the mTOR pathway, represented by 31 unique peptides, and provides insights into several checkpoints involved in regulating cell growth and proliferation. We used it to illuminate the mTOR pathway dynamics at different growth phases of zebrafish PAC2 cell cultures and are currently working on investigating the responses to pharmacological modulators of the mTOR activity, as well as to selected chemicals known to affect fish growth *in vivo*. These experiments will expand our knowledge about the mTOR signalling and growth regulation in fish cells, as well as its role in mediating chemical-induced effects on fish growth.

#### 1.14.P-We026 Combination of Computational New Approach Methods for Enhancing Evidence of Biological Pathway Conservation Across Species

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There is a need for new approach methodologies (NAMs) in chemical risk assessment to help mitigate the ethical and resource burdens of animal-based toxicity testing. With the amount of nonanimal-based methods, approaches, and tools already in existence it is worth investigating how these NAMs can be combined to advance understanding of toxicity. In this case, the value of combining two computational tools, Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) and Genes to Pathways – Species Conservation Analysis (G2P-SCAN) was investigated in relation to supporting cross-species evaluations for predicting chemical susceptibility. G2P-SCAN was used to efficiently gather biological pathway information using gene inputs that represented known molecular targets of chemicals of interest. Network analyses were leveraged with adverse outcome pathway (AOP) information to aid in the prioritization of these mapped pathways. The proteins that comprised these prioritized pathways were then used as a basis for cross-species extrapolation or hypothesis generation for chemical susceptibility using SeqAPASS. In this report, this analysis was described by highlighting the results from three molecular targets: PPARA, ESR1, and GABRA1. The utility and challenges of this combined computational approach were demonstrated. Overall, the weight of evidence to support cross-species susceptibility predictions was enhanced by using biological pathway information. The information gained through this computational approach could ultimately inform chemical risk assessments by enhancing cross-species predictions of chemical susceptibility and potentially by expanding the biologically plausible taxonomic domain of applicability (tDOA) of relevant AOPs.

#### 1.14.P-We027 NAMs in REACH – A Regulatory and Policy Level Perspective

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The main aim of the presentation is to provide a perspective from a regulatory and policy level on how NAMs can be used today in a legislation, reflecting about what is needed to enhance the use of NAMs and where further scientific developments are required. The focus of this contribution is on the EU Regulation REACH.

The presentation is structured in four points:

(1) Opportunities and requirements for using NAMs – current framework: REACH allows already the use of NAMs. Several conditions are attached to their use, inter alia:

- Equivalence of information: NAMs replacing existing information requirements have to provide the information that is requested by the legislation.
- Confidence in NAMs: Regulators need to be able to judge whether the NAM fulfils the information requirement.
- Legal certainty: Registrants should be able to rely on that the use of a specific NAM will satisfy the legal requirements.
- Economic and proportionality considerations

Furthermore, NAMs are used to support, e.g., read-across, grouping and categorisation of chemicals. The presentation will provide examples.

(2) Future developments: The presentation will reflect about future developments that increase the use of NAMs on a short-term (e.g., revision of REACH) and a longer-term timeframe,

(3) Research needs: The presentation will outline research needs. For several endpoints it is still challenging to use NAMs, e.g., for complex human health endpoints.

(4) From science to legislation – or from legislation to research: The last part of the presentation is inspired by discussions the author had at the last SETAC meeting in Copenhagen. Those discussions revealed that academic researchers sometimes have the perception that NAMs developed by them are not taken up by regulators or decision makers. At the same time, regulators and the policy level identify and communicate via different channels needs for scientific developments, but might not always reach the academic research community. The presentation includes a section on possibilities to foster the information exchange between researchers, regulators and the policy level.

#### 1.14.T-01 Enhancing the Quantitative Understanding of an Adverse Outcome Pathway Network for Mitochondrial Dysfunction Using *in vitro* and *in silico* New Approach Methodologies

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Mitochondria are the energy warehouses in eukaryotes. A wide range of chemicals can affect mitochondrial energetic functions. Uncoupling of oxidative phosphorylation (OXPHOS) is one of the most common modes of action of mitochondria toxicants leading to adverse effects of regulatory concern (e.g., growth inhibition). By aligning the adverse outcome pathway (AOP) concept and the 3Rs principles, this study aims to use new approach methodologies (NAMs) to support the development of a quantitative AOP network (qAOPN) and a tiered animal alternative testing strategy for cost-efficient hazard assessment of mitochondrial uncouplers. The AOPN linking mitochondrial uncoupling to growth inhibition in eukaryotes is currently under active development (OECD AOP project 1.92), with one of the linear AOPs being endorsed by WPHA/WNT

(<https://aopwiki.org/aops/263>). To quantify key events (KEs) in this AOPN, a suite of high-throughput *in vitro* bioassays was conducted with the zebrafish liver (ZF-L) cell-line, after a time-course exposure (2, 6, 12, 24, 48, and 72 h) to nine concentrations (0.001–100 µM) of the model uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP). The KEs measured include: mitochondrial membrane potential (MMP), ATP level, calcium signalling, cytotoxicity (metabolic activity and cell membrane integrity), and cell proliferation. In addition, chemical analysis was performed of CCCP in the cells and exposure media. Temporal and concentration dependent responses of ZF-L cells to CCCP were observed for the KEs of interest as early as 2 h of exposure. The uptake and metabolism of the different CCCP concentrations corresponded well to the temporal effect response

observed in the cells. The most sensitive and responsive (2 h) KE was uncoupling of OXPHOS as indicated by decreased MMP. A decrease in ATP was observed after 6 h of exposure, followed by a clear concentration-dependent decrease in cell proliferation. There was no apparent change in cytosolic calcium signalling until 6-12 h of exposure. A slight increase in cell death was also observed when exposure duration increased. The data were further used to construct quantitative KE relationships in an AOPN. The coefficients for molecular initiating- and several of the key events were statistically significant in the AOPN. This study has demonstrated the use of NAMs for qAOPN development. Current work has supplied novel causal knowledge and a new workflow for qAOPN construction for future studies.

#### 1.14.T-02 AOP-Based Evidence from Chicken Embryos and the Chicken Hepatic LMH Cell Line that Bisphenol A and Ethinyl Estradiol Induce Dysregulation of Bile Acid Homeostasis in Birds

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Bile acids play an important role in cholesterol metabolism and secretion of endogenous and xenobiotic compounds. Concentration of bile acid is tightly regulated by nuclear receptors and transporters. One adverse outcome pathway (AOP #27, <https://aopwiki.org/aops/27>) documents how inhibition of the bile salt export pump (BSEP) can interfere with bile acid homeostasis and lead to cholestasis. Previous studies have found that environmental chemicals can impair bile acid homeostasis and cause cholestasis in birds. In the present study we measure several endpoints along the BSEP inhibition AOP following exposure to BPA and EE2 in two avian model systems 1) an early life stage model, the chicken embryo, and 2) an *in vitro* model, immortalized chicken hepatic cells, LMH, cultured as spheroids. The objectives were 1) to determine if BPA and EE2 are probable inducers of liver cholestasis in birds, based on AOP-based evidence, and 2) investigate if spheroid LMH cells could be a suitable model to screen for cholestasis-inducing chemicals for avian toxicity testing. Embryos were exposed via egg injection prior to incubation and data were collected at two developmental stages, mid-incubation (embryonic day [ED] 11) and term (ED20). Changes in expression of bile acid genes and bile acid concentrations were measured at both time points. Morphometric measurements were recorded at ED20. LMH spheroids were treated with BPA and EE2 for 8, 24, and 48 h. Changes in gene expression were evaluated at all three time points and bile acid concentration at 24 and 48 h. Both BPA and EE2 resulted in modulation of bile acid genes, including downregulation of BSEP, in mid-incubation livers and LMH spheroids at 24 h. The magnitude of change in gene expression was greater in mid-incubation livers and LMH spheroids at 24 h compared to term livers and LMH spheroids at 48 h. BPA and EE2 increased bile acid concentration in term livers and LMH spheroids at 48 h. Exposure to BPA (50 µg/g) and EE2 (25 and 50µg/g) significantly decreased gallbladder mass and increased liver mass in ED20 embryos. In summary, we found that BPA and EE2 downregulated BSEP mRNA expression, and increased bile acid levels in hepatocytes. Based on the BSEP AOP, this evidence suggests that these compounds could induce cholestasis in birds. Further, these results suggest that LMH cells cultured as 3D spheroids may be a suitable *in vitro* model to screen chemicals that impair bile acid homeostasis.

#### 1.14.T-03 Evaluation of Thyroid Hormone System Disrupting Potential of Resorcinol in Fish Using an AOP-Based Approach

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An increasing number of pollutants are reported to interfere with the thyroid hormone system, raising concerns for both human and environmental health. Recently, resorcinol was brought up as a substance of very high concern due to thyroid hormone system disruption (THSD). It is used for a variety of purposes, such as applications in the rubber industry, but also as a substance in pharmaceuticals. There is *in vitro* evidence that resorcinol can inhibit thyroperoxidase (TPO), an enzyme crucial in thyroid hormone (THs) synthesis. Furthermore, resorcinol exposure has been linked to hypothyroidism in humans. Evidence linking the compound to THSD in the environment is currently lacking. In the current study, the THSD potential of resorcinol *in vitro* and *in vivo*, in the zebrafish embryo model, was assessed using the adverse outcome (AOP) conceptual framework. AOPs linking THSD to impaired swim bladder inflation and eye development in fish have been previously developed. *In vitro* data were gathered to predict the possible molecular initiating events (MIEs), and confirmed that resorcinol is a TPO inhibitor. Additionally, resorcinol was identified to be a thyroid receptor (TR) antagonist and a transthyretin (TTR) displacer. TH levels were assessed following resorcinol exposure in zebrafish embryos. Whole-body thyroxine (T4), the main synthesised TH, levels were significantly reduced, showing that resorcinol is capable of inhibiting TPO *in vivo*. Resorcinol exposure significantly impaired swim bladder inflation. T4 supplementation could not rescue the effect on the swim bladder, showing that this effect is not a result of TPO inhibition, and is likely attributable to one of the other identified THSD mechanisms, such as TR antagonism and TTR displacement. Swimming performance in embryos with uninflated swim bladders was significantly impaired and the effect depended on the resorcinol concentration. Additional analyses, including gene transcription analysis and eye histology, are being performed, which will aid in further elucidating the effect of resorcinol both on the thyroid hormone system and THSD-related endpoints *in vivo*. The current study is valuable for the development of THSD screening methods, as it illustrates how an AOP-based approach can be used to consider the contributions of multiple mechanisms when evaluating the THSD potential of a chemical.

The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.

#### 1.14.T-04 Unraveling Molecular Mechanisms of Alkyl Sulfonic Acid PFAS-Dependent Hyperactivity in Larval Zebrafish Using Gene-Editing

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Per- and polyfluorinated alkyl substances (PFAS) are a diverse class of synthetic chemicals widely used in industrial manufacturing. We previously showed that exposure to alkyl sulfonic acid PFAS (perfluorooctanesulfonic acid (PFOS) or perfluorohexanesulfonic acid (PFHxS)) caused dark-phase hyperactivity in larval zebrafish. To confirm that PFAS-dependent behavioral effects result from a developmental perturbation, swimming behavior was assessed in 5-day post fertilization (dpf) larvae following either developmental (1-4 dpf) or acute (5 dpf) exposure to 0.43-7.86  $\mu$ M PFOS, 7.87-120  $\mu$ M PFHxS, or 0.4% dimethyl sulfoxide (DMSO). Relative to DMSO, dark phase hyperactivity occurred both developmentally and acutely in PFAS-exposed larvae. In contrast, visual startle response hyperactivity only occurred following developmental exposure. A wash-out study was performed where developmental exposure to PFOS or PFHxS from 1-4 dpf triggered irreversible concentration-dependent visual startle response hyperactivity at 5-8 dpf. Peroxisome proliferator-activated receptors (ppara, ppard, pparg) were identified as putative upstream regulators. To test the hypothesis that ppars are required for PFAS-dependent visual startle response hyperactivity, CRISPR/Cas9-based gene editing was used to knockdown ppara/ab, pparda/db, or pparg at day 0. Knockdown larvae were exposed to 7.86  $\mu$ M PFOS, 80  $\mu$ M PFHxS, or 0.4% DMSO from 1-4 dpf and locomotor activity was assessed at 5 dpf. ppara/ab or pparg knockdown did not affect the ability of PFOS or PFHxS to provoke visual startle response hyperactivity. In contrast, pparda/db knockdown blunted PFOS and PFHxS-dependent hyperactivity as compared to the exposed negative control. Orthogonal confirmation using the ppard antagonist GSK3787 confirmed that ppard is required for PFOS-dependent visual startle hyperactivity. This work identified two distinct phenomena. One, exposure to PFOS or PFHxS causes acute and transient dark-phase hyperactivity. Two, developmental, but not acute, exposure to the same chemicals triggers persistent visual startle response hyperactivity that requires the activity of ppard. Taken together, we identified a novel adverse outcome pathway for hyperactivity in the visual startle response elicited by structurally similar PFAS. More broadly, this work shows how gene editing can be used for rapid hypothesis testing in larval zebrafish.

#### 1.14.P Using New Approach Methods to Move from Descriptive to Mechanistic Ecotoxicology

##### 1.14.P-We028 *In vitro* Screening of UV-Stabilizers and UVFilters: Cytotoxicity, CYP1A Activity, and mRNA Expression in an Immortalized Embryonic Double-Crested Cormorant Cell Line

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Ultraviolet (UV) stabilizers and UV filters are added to industrial and personal care products to absorb UV rays and prevent photooxidation. Some of these compounds have been listed as substances of concern by the European Chemical Agency due to their persistence in the environment and large production volume. UV stabilizers and UV filters have been detected in a range of environmental matrices, including high trophic level bird species (e.g., double-crested cormorant). Little is known about the toxicological properties of these compounds, especially in birds. Previously, we established and characterized an immortalized embryonic double-crested cormorant hepatic cell line, DCH22, for chemical screening. In the present study, DCH22 cells, cultured as 3-dimensional (3D) spheroids, were exposed to four UV stabilizers (UV-328, UV-329, UV-9, UV-P) and a UV filter (BP-3) at nominal concentrations of 0.1 to 1000  $\mu$ M to determine cell viability, CYP1A activity and changes in mRNA expression. Cell viability and CYP1A activity were measured using intracellular ATP concentration and ethoxyresorufin-O-deethylase (EROD) activity. A customized species-specific PCR array, EcoToxChip, comprising 384 genes, will be used to determine changes in mRNA expression. The UV stabilizers and UV filter elicited similar effects on cell viability with LC50 values ranging from 145 to 208  $\mu$ M. UV-P and BP-3 exposure resulted in a concentration-dependent increase in CYP1A activity with BP3 inducing a greater response than UV-P. UV-328 and UV-329 did not induce CYP1A activity in DCH22 spheroids. Concentration-dependent (0.1 to 100  $\mu$ M) changes in mRNA expression will be evaluated for the five compounds and the dose-response modelling program PROAST (RIVM) will be used to determine benchmark doses. The CYP1A activity and gene expression data will provide information on the mechanism of toxicity for each chemical. Given the increased use and prevalence of these compounds, these findings will generate much needed toxicity data in a wildlife avian species.

##### 1.14.P-We029 Directional Reactive Model Predicting *in silico* Ligand-Aryl Hydrocarbon Receptor Activity

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The protein receptor as a transcription factor mediates the enzyme regulation and determines how the cells in organism adapt drugs or metabolize xenobiotics. Since the activation of receptors is a ligand-specific and cell-type specific process, understanding of how the physicochemical factors configure the environment for ligand-receptor binding is a key to predictive assessment of drug efficacy or toxicity of environmental pollutants. Here we show that the binding affinity of homologues of chrysene to the aryl hydrocarbon receptor is dominantly influenced by dipole moment-aligned reaction of ligands with charge states of amino acids forming the AhR. We propose the directional reactivity factor as a mechanistic prediction indicator linking molecular ligand-receptor binding to in vitro toxicity. The DRF accurately estimated toxic potency of a set of similarly structured polycyclic aromatic hydrocarbons, as confirmed by H4IIE-*luc* bioassay.

There are several “new findings” from the present work:

- This study is the first to apply a novel approach, based on mechanistic prediction of binding of ligands to their respective protein receptors, by calculating physicochemical properties in PAHs and their binding to the AhR. Of note is the interpretation and utilization of their properties of this class of toxicants that were not clear to date.
- The present article summarizes limitations of current models to predict toxic potencies of similarly structured chemicals, either drugs or contaminants and presents a novel model and perspective for more accurate prediction of binding of drugs or contaminants to receptors. The expansion and development of this model not only raised new and challenging topics in approaches to predict toxicity of contaminants, but also can help improve other existing models.
- This is the first approach to assess interactions between toxicants and receptors based on interplay of physicochemical properties as determined by the first principles calculation and validated by use of synchrotron-radiation X-ray spectroscopy. Using this novel method, it will be possible to classify species-specific toxic potencies of chemicals in different environments. Our experiment-free first principles approach provides an analytical framework for predicting molecular bioactivity in silico and complements conventional observation-based bioassays.

#### **1.14.P-We030 Development of Adverse Outcome Pathway Network Based on Molecular Initiating Event Identification Using Molecular Docking Simulation: A Case Study with Additive Chemicals in Plastics**

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Computational molecular docking simulations can rapid screening of numerous chemicals by predicting which ligands bind to target proteins, and discover molecular initiating events (MIEs) in an Adverse Outcome Pathway (AOP) framework. There is an increased need to identify potential toxicity of additive chemicals in plastics. In this study, (1) AOP networks (AOPNs) with nuclear receptors as MIEs were developed using the Comparative Toxicogenomics Database (CTD), and (2) additive chemicals in plastics were prioritized through molecular docking simulations for potential MIEs. From this, (3) we developed an approach to identify the potential AOP based on CTD and molecular docking simulation. A focus was made on EDC-relevant nuclear receptors related response. As stepwise, the largest EDC-relevant AOPN associated with nuclear receptors was compiled from the AOP Wiki, consisting of 19 Key Events (KEs), 5 AOPs, and 2 Adverse Outcomes. Potential biomarkers were derived by combining the known diseases and toxicity mechanisms of each receptor and each ligand using CTD. We generated 947 CGPD tetramers for “Carcinoma, Hepatocellular” associated with 3 genes (PPARG, FXR, and LXRB), and 4 CGPD tetramers for “adenoma, the liver cell” associated with a gene (CAR). Collectively, we identified the strengthened MIE related to CAR and the potential MIEs related to PPARG, FXR, and LXRB. Molecular docking analysis was performed using 223 plastic additives on 17 human nuclear receptors. According to the binding affinity values, plastic additives were prioritized as potential MIEs of nuclear receptors by type of use. These results highlight the applicability of molecular docking to screen priority chemicals as well as the identification of toxicity mechanisms of potentially hazardous chemicals. The approach proposed in this study is expected to provide insight into toxicity mechanisms and chemical-gene interactions in chemical risk assessment.

#### **1.14.P-We031 One Substance, One Assessment – Unachievable Ambition or an Opportunity for NAMs Not to be Missed?**

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As a part of the EU's Zero Pollution Strategy within the Chemicals Strategy for Sustainability there is a proposal for the implementation of a “One Substance, One Assessment” approach. Such an aim is ambitious given the divergence of regulatory frameworks, data requirements, exposure scenarios and underpinning guidance in the various sectors under which a chemical may be registered for different uses (e.g., general chemicals, biocides, pesticides, pharmaceuticals etc).

All forms of risk assessment should start with problem formulation as a central principle acknowledged for over 30 years. The current package of *in vivo* ecotoxicology studies is a group of test methods designed to generate data to answer a problem that has generically been formulated by the predefined protection goals, surrogate receptors and sector specific associated guidance. In practice the problem formulation is rarely revisited in the risk assessment in a defined manner. As a consequence, many nonanimal methods or New Approach Methodologies (NAMs) proposed for use in risk assessment tend to focus on, or are judged as, direct replacement of *in vivo* testing, rather than centering on the aspects of the ‘problem’ they can define or address.

When considering NAMs it is important to revisit the problem formulation phase of the risk assessment. This clarifies what information can be provided by NAMs to address the case specific problem – thereby contributing to an overall reduction in the

vertebrate testing burden. Here we present a more chemical-specific problem formulation that can drive the robust implementation of NAMs within risk assessment. Leveraging problem formulation to drive the early generation of nonanimal data and NAMs (e.g., physical chemical properties, environmental fate as well as *in vitro* and *in silico* approaches) enables the design of substance specific approaches (i.e., meeting the objective of the One Substance, One Assessment ambition) whilst reducing our reliance on *in vivo* vertebrate testing.

This concept will demonstrate how building on well-established principles of risk assessment, can enable modern scientific technology and techniques to deliver the better use of NAMs providing robust environmental safety assessments.

#### **1.14.P-We032 Exploring the Taxonomic Domain of Applicability (tDOA) of Thyroid Hormone System Disruption using the Adverse Outcome Pathway Framework**

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Multiple studies have associated chemical exposures with endocrine disruption, and specifically thyroid hormone system disruption (THSD), in humans and wildlife. Current regulatory efforts focus on developing fast and efficient screening methods to identify thyroid hormone system disrupting compounds in the environment. These efforts comprise the substitution of animal-based assays, mainly rodent studies, by new approach methodologies (NAMs) such as zebrafish or amphibian embryo assays and thus increase future needs for cross-species extrapolation. Adverse outcome pathways (AOPs) can support this need by providing causal linkages from molecular initiating events (MIEs; describing the interaction between a chemical and a molecular target) to adverse outcomes (AOs; on individual or population level) via key events (KEs; describing measurable, altered biological states).

Different AOPs for THSD have been developed which are, in most cases, applicable to a single taxon. They do, however, share multiple KEs and thus form a THSD AOP network that connects, e.g., AOPs developed in fish with AOPs described in mammals. Making use of this network, we evaluated whether paths leading, for example, to impaired learning and memory in mammals, are potentially applicable to other vertebrate taxa. In doing so, we aimed to identify paths with a high potential for cross-species extrapolation that could further support the development of tailored, alternative (non-mammalian) test methods.

Assessing the taxonomic domain of applicability (tDOA) of MIEs and AOs in the THSD network was achieved by means of a literature study. In this study a special focus was on the link between MIEs and AOs with altered thyroid hormone levels as these present key elements in the THSD network, linking almost all AOPs with each other. Our meta-analysis suggests that a majority of MIEs is applicable across taxa. Further, we found that paths in the network leading, for example, to adverse neurodevelopmental outcomes, possess a high potential for cross-species applicability, while paths leading to thyroid cancer are most likely applicable to rats only. Based on this analysis we are now able to prioritise paths suitable for cross-species extrapolation and highlight the potential of using data from non-mammalian vertebrate classes to predict THSD-induced effects in mammals, including humans, and vice versa.

#### **1.14.P-We033 Application of 'Omics for Identification of Mode of Action-Specific Molecular Fingerprints and Protein Biomarkers Induced by the Fungicide Carbendazim in Zebrafish Embryos**

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Chemical substances, including fungicides, that are introduced to the ecosystem in a targeted manner can adversely affect nontarget organisms inhabiting the ecosystem. Currently, regulatory agencies mandate a series of studies to evaluate environmental side effects, including analysis of bioaccumulation, environmental fate and toxicity in various model organisms. However, these studies do not capture the early cellular responses to the chemical stressors at the molecular level. The application of recent OMICS technologies enables sensitive and global identification of molecular changes at the level of RNA (transcriptomics) and proteins (proteomics). According to the 2022 FRAC (Fungicide Resistance Action Committee) classification, Carbendazim is classified as an inhibitor of the cytoskeleton and motor proteins synthesis in fungi. The aim of this work was to predict the early responses induced by Carbendazim in zebrafish embryos in terms of changes in gene expression supported by proteomic profiling of the same sample. Zebrafish embryos were exposed to sublethal concentrations of carbendazim for 96 h followed by simultaneous RNA and protein extraction from the respective samples. To further analyse effects induced by carbendazim in transcriptomic and proteomic profiles, RNA-Seq (transcriptome) and mass spectrometry-based proteomics (proteome) analysis were applied. The resulting molecular fingerprints and protein biomarkers will provide the basis for developing a mode of action (MoA)-specific screening approach for active substance precursors under development as well as for monitoring environmental samples to detect early responses to environmental contaminants. In-depth knowledge of molecular changes induced by active substances with different MoA and linkage to phenotypic and population effects will significantly facilitate the classification of active substances in terms of their environmental side effects.

#### 1.14.P-We034 Exploring the Molecular Basis of Transient versus Persistent Neurotoxic Effects Using a Multi-Omics Approach in the Zebrafish Model

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Neurotoxicity occurs when exposure to toxic substances alters the normal functioning of the nervous system. A common way of assessing functional significance of changes in the nervous system is the measurement of an organism's behavioral output, as this reflects the combined action of neuronal, neuroendocrine, and neuromuscular signals. Behavior assessment in early life stages of zebrafish *Danio rerio* is time- and cost-effective, due to its small body size, well-developed testing methodology, and commercial availability of relevant instrumentation. However, it remains unclear, to what extent the observed behavioral effects reflect specific changes in the nervous system, and whether these alterations are transient or irreversible. Therefore, to investigate mechanistic links that could help in the interpretation of behavioral observations, we analyzed zebrafish larvae behavior concomitantly with molecular, cellular and structural changes in the nervous system. For this, zebrafish larvae are exposed to a set of neurotoxic substances and then depurated in clean water to assess recovery potential. We measured (i) functional changes through behavior assessment, (ii) cellular and structural changes such as synaptic connections, neuronal fibers, and muscular structure by using immunohistochemical markers and (iii) molecular changes by transcriptomics, proteomics and metabolomics focused on molecules relevant to the functioning of nervous system, such as selected receptors, enzymes and neurotransmitters. Previously, we demonstrated that most insecticide-induced behavioral changes in zebrafish larvae are of transient nature, even in the presence of concomitant structural changes in motoneuron outgrowth or muscles. We have now investigated the persistence of behavioral changes upon exposure to other neuroactive/neurotoxic chemicals (e.g., the opioid fentanyl citrate, the serotonin receptor agonist buspirone and the plastic chemical bisphenol A) and found that most behavioral effects partially or fully recovered as well, albeit at different rates. With the currently ongoing molecular analyzes we aim to identify molecular markers that could be predictive of transiency or persistency of neurotoxic effects. These insights will aid interpretation of test results obtained in zebrafish behavior assays and will be used to explore whether and how to include behavioral testing into hazard assessment of neurotoxic substances.

#### 1.14.P-We035 Molecular Signature of Thyroid Hormone System Disrupting Chemicals

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Endocrine disrupting chemicals pose a threat to both humans and wildlife. The hypothalamic-pituitary-thyroid axis has been identified as an area with large knowledge gaps and insufficient test guidelines, not at least when it comes to early life stages. Aiming to evaluate and discover molecular key events for thyroid hormone system disruption, we used targeted quantification of 12 thyroid hormones (TH) and metabolites as well as untargeted metabolomics to investigate the molecular fingerprint of one model- and three suspected thyroid hormone system disrupting chemicals (THSDC). Propylthiouracil (PTU) is an antithyroid drug used to treat hyperthyroidism, which has been extensively used as a model THSDC. The brominated flame retardant tetrabromobisphenol-A (TBBPA), and the two pesticides pyraclostrobin and amitrole are compounds that have shown thyroid activity either *in vitro* or in *in vivo* experiments with other vertebrate species. To target the perinatal life stage, pregnant C57bl/6 mice were exposed from gestational day 7 to postnatal day 14, resulting in *in utero* and lactational exposure of the pups. Blood was then sampled from both mothers and pups and enriched for analysis in the targeted and non-targeted workflow.

TH metabolism was significantly affected by PTU, amitrole, and pyraclostrobin, but not TBBPA. Interestingly the effects on the TH pathway were not similar across compounds. For instance, thyroxine (T4) and 3,3',5'-triiodothyronine (rT3) were decreased by PTU but increased by pyraclostrobin in both mothers and pups. Iodothyronine (T1) was decreased by PTU in mothers but increased by amitrole. Metabolomics analyses detected more than 10 000 endogenous metabolites in mothers and pups. Analyses are currently ongoing, but at least 6 metabolites were found to be significantly affected by both amitrole, pyraclostrobin and PTU in pups. These biomolecules are potential new quantifiable key events for determining thyroid hormone system disruption at the perinatal life stage. The results show that THSDCs may have different underlying mechanisms of toxicity and affect for instance thyroid hormone metabolic pathways at different points. This underlines the importance of not only looking at the most commonly measured thyroid hormones T3 and T4. Untargeted metabolomics is furthermore a valuable technique in identifying new approach methods to expand the repertoire of key event used to assess THSDC

#### 1.14.P-We036 Does the Zebrafish Microbiome Bioactivate PFOS Precursors?

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Chemical exposure can alter host-associated microbiomes. In turn, host-associated microbiomes harbor the capacity to modify chemical structures and form biotransformation products. Whether these changes cause adverse effects at the level of the host remains an open question. We previously showed that exposure to PFOS evokes hyperactivity in 5-day postfertilization (dpf) zebrafish. Although PFOS has been phased out of use, it has been replaced by larger, branched PFAS that are widely detected in the environment and can undergo biotransformation to form PFOS. We hypothesized that, similar to 5 dpf zebrafish, PFOS-dependent hyperactivity occurs in older larvae and that the microbiome can bioactivate PFOS precursors to generate the active metabolite PFOS. To test our hypothesis, zebrafish embryos were exposed to PFOS (0.28-5  $\mu$ M), the PFOS precursors N-EtFOSA (0.07-1.25  $\mu$ M) or PFOSAmS (0.83-15  $\mu$ M), or 0.1% DMSO from 5-6 dpf. Chemical was removed at 7 dpf and

swimming behavior was assessed at 8 dpf using a light-dark transition test. Similar to 5 dpf larvae, exposure to PFOS resulted in light- and dark-phase and visual startle response hyperactivity at 8 dpf. In contrast, exposure to N-EtFOSA or PFOSAmS triggered light- and dark-phase and visual startle response hypoactivity. Concentrations of the PFOS precursors and the metabolite PFOS were analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for target quantification. To identify the enrichment of microorganisms that harbor the potential to biotransform N-EtFOSA or PFOSAmS into PFOS, gut dissections were performed in 8 dpf larvae exposed to 5  $\mu$ M PFOS, 0.7  $\mu$ M N-EtFOSA, 8.4  $\mu$ M PFOSAmS, or 0.1% DMSO and used for 16S rRNA gene amplicon sequencing and metagenomic sequencing to describe changes in community structure. These results show that larval exposure to PFAS compounds results in different phenotypic outcomes. Future work will pair the developed analytical approach with metagenomic sequencing to identify species-specific enzymes that are capable of bioactivating PFAS precursors to form PFOS. Collectively, this work seeks to address the degree to which the host-associated microbiome bioactivates widely occurring environmental chemicals.

#### **1.14.P-We037 A New Approach Methodology (NAM) for Risk Assessment of SSbD Nanomaterials Based on Adverse Outcome Pathways Approach, Real Exposure Scenarios and Relevant Hazard Testing**

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The use of new approach methodologies (NAMs) for the hazard assessment of chemicals, including nanoparticles (NPs) and nano-enabled products (NEPs), is rapidly increasing, due also to the request of the European Commission to promote new tools for the development of safe and sustainable by design (SSbD) materials. NAMs include *in silico*, *in vitro* and adverse outcome pathways (AOPs) testing strategies as alternative methods to animal testing. In this work, we provided a NAMs-based framework for the risk assessment of NPs, designed according to SSbD approach. The NAM we propose integrates the AOP perspective, real human exposure assessment and novel hazard testing approaches. First, NPs were assessed for their safety: NPs physicochemical properties were linked to the mechanistic aspects of their biological outcomes, using A549 human lung cells as target systems (Tier 1, *molecular*, and Tier 2, *cellular*, level of a given AOP). Comparative *in vitro* cytotoxicity tests were performed to define and measure representative biomarkers of key events (KE) along possible NPs-driven AOPs, with oxidative stress, inflammatory and genotoxic pathways being the main players. For realistic exposure scenarios, particulate matter (PM) mass concentration data from environmental monitoring campaign at a manufacturing site, during an industrial process to produce NEPs of interest, were gathered. Aerosol measurements (e.g., NPs size, concentrations, and effective density in air), were then used as inputs to calculate the alveolar retained doses through MPPD modelling. The MPPD doses, were then translated in real occupational monthly or yearly human exposure doses and then tested in an *in vitro* co-culture model representative of the lung alveolar space (A549 + THP1-derived macrophages cells). Exposures were performed at the air liquid interface (ALI), using the Vitrocell® Cloud  $\alpha$ -12 system, to mimic actual inhalation and deposition of the NPs at the manufacturing site. Preliminary hazard endpoints evaluated were cell viability and inflammatory responses. This integrated approach represents a NAM that integrates a procedure to calculate the exposure doses for humans and the potential hazard resulting from the exposure *in vitro* complex models (Tier 3 according to AOPs). The results obtained, due to the application of NAMs, allow for more reliable and realistic interpretation of the hazard of new SSbD nanomaterials.

#### **1.14.P-We038 Development of Potential Adverse Outcome Pathway for Neurotoxicity From Toxicogenomics Data Using Benchmark Dose Modeling Approach: Case Study on Valproic Acid**

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Benchmark Dose (BMD) is a value calculated by a statistical method from a dose-response model and represents a certain level of pre-determined effect compared to the control group. valproic acid (VPA) is a drug used to treat epilepsy and bipolar disorder. Although rare, side effects such as spina bifida and neural tube obstruction may occur. Recent studies have shown that VPA affects cell growth, differentiation, apoptosis, and immunogenicity. In this study, by using human and rat 'omics data for VPA collected from Open TG-GATES, an open toxicogenomics database, BMD values were analyzed to determine the key event (KE) and its sensitivity to develop quantitative adverse outcome pathway (AOP). Differentially expressed genes (DEGs) were analyzed from 'omics data using BMDExpress and Ingenuity Pathway Analysis (IPA), and major toxicity pathways were identified through toxicity pathway analysis based on DEGs. Then, BMD values were calculated and mapped onto DEGs and pathways to identify the important processes initiated at these values. Disease and phenotype data were collected from the AOP Wiki, Comparative Toxicogenomic Database (CTD, <http://ctdbase.org/>), and literature review to build the AOP. Finally, we suggest an AOP for neurotoxicity based on toxicogenomics data using benchmark dose modeling approach. This study showed the use of 'omics data in AOP development and the possibility of using BMD in quantitative AOP development.

## **Track 2: Ecotoxicology Becomes Stress Ecology: From Populations to Ecosystems and Landscapes**

### **2.01 Advantages of Using Lab and Field Collected Invertebrates and *in situ* Studies in Ecotoxicology: Challenges and Opportunities**

## 2.01.P-Th099 Environmental Concentrations of Carbaryl and Fenitrothion Alter Highly Conserved Biological Responses: From *D. magna* to Fish Species

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Carbaryl and fenitrothion are two insecticides sharing a common mode of action, the inhibition of the acetylcholinesterase (AChE) activity. Their use is now regulated or banned in different countries, and the environmental levels of both compounds in aquatic ecosystems have decreased to the range of pg/L to ng/L. As these concentrations are below the non-observed-adverse-effect concentrations (NOAEC) for AChE inhibition reported for both compounds in aquatic organisms, there is a general agreement that the current levels of these two chemicals are safe for aquatic organisms. In this study we have exposed *Daphnia magna* and two fish species, zebrafish and medaka, to concentrations of carbaryl and fenitrothion under their NOAECs for 24-h, and the effects on heart rate (HR), basal locomotor activity (BLA), visual motor response (VMR), startle response (SR) and its habituation have been evaluated. Both pesticides increased the HR in the three selected model organisms, although the intensity of this effect was chemical-, concentration- and organism-dependent. The exposure to both pesticides also led to a decrease in BLA and an increase in VMR in all three species, although this effect was only significant in zebrafish larvae. For SR and its habituation, the response profile was more species- and concentration-specific. The results presented in this manuscript demonstrate that concentrations of carbaryl and fenitrothion well below their respective NOAECs induce tachycardia and the impairment of ecologically relevant behaviors in phylogenetically distinct aquatic model organisms, both invertebrates and vertebrates, emphasizing the need to include this range of concentrations in the environmental risk assessment.

## 2.01.P-Th100 Establishment of Chronic Toxicity Testing with *Cloeon Dipterum* Including Transcriptomics-Based Molecular Profiling

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Aquatic larvae of the so-called EPT-taxa are frequently exposed to multiple contaminants and are shown to be highly sensitive. In Europe we are still lacking standardized test systems with more sensitive species, particular for chronic tests. We adapted a test system for chronic toxicity tests with the lentic mayfly species *Cloeon dipterum* and included transcriptomics-based molecular profiling.

Two chronic toxicity tests with field-collected larvae in an early development stage were performed; in both tests, Fipronil was used as test item in environmentally relevant concentrations (0.037 – 0.60 µg/L). The tests were conducted in glass beakers under conditions of standing water. During the tests, the grazing larvae were fed with *Navicula* grown on small tiles and carrots. The exposure was semistatic and test solutions and food were replaced twice a week. At each media renewal the development stages of the larvae were determined. As endpoints the larval development throughout the test, emergence and mortality were examined. In each test the concentration of Fipronil was determined by LC-MS/MS.

The long-term exposure test was conducted until all individuals had emerged or died (38 days). Emerged female individuals were sampled for further transcriptomic analysis. For the sublethal endpoint larval development statistical evaluations were performed for several time points. After a test duration of 7 days an EC50 of 0.70 mg/L was determined. For this test transcriptomics revealed no significantly differentially expressed genes when comparing treatments and control. Based on these results, we decided to repeat the test with an adapted test design.

The short-term exposure test was conducted for 7 days and transcriptomic analysis was performed with the larvae. For the endpoint larval development an EC10 of 0.029 mg/L was determined. Indeed, with this test setup, significantly differentially expressed genes (DEGs) were detected in the larvae using transcriptomics. We observed a concentration-dependent increase in the numbers of DEGs as well as strong positive correlation for their expression changes when comparing the different exposure concentrations.

We established a system for chronic toxicity tests with the lentic mayfly species *C. dipterum* successfully. Larval development was pointed out as an appropriate endpoint. Furthermore, transcriptomics were successfully applied to this model organism observing concentration-dependent gene expression changes.

## 2.01.P-Th101 Uncovering Metabolic Disturbances in Chitin Metabolism after Exposure to Teflubenzuron and Linking Them to Phenotypical Effects in *Daphnia magna*

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In order to grow, reproduce, and survive, arthropods need to shed their cuticle (exoskeleton) periodically through molting. In order to molt, arthropods depend on the formation of a new, larger cuticle stable to support muscular contractions during the



molting process. As chitin is a major constituent of the arthropod cuticle, its synthesis is crucial and has been exploited as a target for control of unwanted arthropods by development of compounds such as chitin synthesis inhibitors (CSIs). We investigated the effects of the chitin synthesis inhibitor teflubenzuron (TEF) on selected metabolites of the chitin biosynthesis – and degradation pathway, as well as on molting and associated mortality. We aimed to identify responsive metabolites in the pathway and find possible metabolic markers to predict adverse phenotypical effects. To achieve our objectives, we exposed *Daphnia magna* to TEF (0.1–8 µg/L) for 48 h. After 24 h of exposure, we sampled *D. magna* for subsequent metabolite extraction and quantitative LC/MS analysis of 6 selected metabolites involved in chitin synthesis- and degradation.

We observed a dose dependent decrease in Trehalose (Tre) and increase in Glucose (Glc) levels, as well as nonmonotonic responses of Glucosamine (GlcN) and *N*-Acetylglucosamine (GlcNAc). The observed 24 h benchmark dose concentrations (BMCs, based on zSD Benchmark response) for the change in Tre, Glc, and GlcNAc (0.19, 0.53, and 0.44 µg/L, respectively), were found to be lower than both 24 h and 48 h BMCs for molting (2.64 and 2.63 µg/L, respectively) and survival (3.16 and 0.95 µg/L, respectively), indicating that metabolic modifications occurred prior to and at lower concentrations of TEF than adverse phenotypical (apical) effects. Alteration in levels of metabolites involved in both chitin synthesis and degradation suggest that multiple pathways, that are key to chitin synthesis and ultimately chitin integrity, are affected by exposure to TEF. In summary, quantified carbohydrates involved in chitin synthesis and degradation were found to be more sensitive endpoints than phenotypical effects and establish themselves earlier than the onset of molting disturbances or mortality.

## **2.01.T-01 The Population Development of Splash Pool Organisms under Copper and Predation Risk Exposure in Field Conditions**

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In nature, organisms must cope with multiple biotic and abiotic stressors and anthropogenic contaminants. Copepods are one of the most abundant organisms and a crucial link in the aquatic food web. Especially coastal copepods are exposed to anthropogenic stressors such as copper. Copper (Cu) is an essential metal, but it becomes toxic at elevated concentrations and is used as a biocide in agriculture and as an antifoulant in aquaculture.

Natural factors can alter organisms' responses to contaminants. Predation risk affects population dynamics and interferes with their responses to Cu. Copepods often contain the carotenoid pigment astaxanthin – an antioxidant that lessens the impact of Cu, but increases the risk of visual predation. Contaminants' toxicity also depends on the physicochemical environment. In the intertidal and spray zone, conditions vary from optimal to hostile. Intertidal organisms, such as the harpacticoid copepod *Tigriopus brevicornis*, are adapted to cope with such extreme environments. Despite their hardiness, we saw potentiating effects of predation risk on copper effects on development in the lab, even at sub-lethal Cu concentrations. Here we tested whether these findings were detectable in a realistic field setting. We initiated 36 outdoor mesocosms and exposed them to predation risk (fish kairomones), Cu (10 µg L<sup>-1</sup>), combined, and control. Each month, we quantified the population composition and size of copepods and other mesocosm inhabitants, and measured copepod's pigmentation and stable isotope composition.

Treatments had weak effects on the copepod population but a positive effect on nonbiting chironomids, which seem to thrive in the combined stressor treatment. In addition to the treatments, all inhabitants experienced high and fluctuating temperatures and salinities. Despite these extreme conditions, copepods were more pigmented with kairomones but not in combination with Cu. Our study shows the importance of studying contaminant effects in an environmentally realistic setting. Natural factors can interfere with and potentially mask long-term contaminant exposure's effects. However, even if temperature and salinity effects might be a more immediate threat to coastal copepod populations due to the ongoing climate crisis, natural and anthropogenic stressors are still detectable in natural populations and potentially influence the ability of copepods and other organisms to establish stable populations.

## **2.01.T-02 The Impact of the Anti-Sea Lice Chemotherapeutant, Hydrogen Peroxide, on Three Nontarget Crustacean Species**

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The expansion of Atlantic salmon aquaculture in Norway has raised concerns about its impact on the marine environment, including negative effects on wild salmonid populations, the release of nutrients and chemical pollutants, as well as the spread of parasites such as the sea lice. To control the sea lice infestations in the salmonid farms, strict regulations have been put in place. To comply with these regulations, the industry has relied on chemotherapeutants. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a delousing agent, has the potential to negatively impact other species other than sea lice. Wild nontarget populations, living in the vicinity of aquaculture facilities, are especially at risk. Moreover, there is a growing need to carry out toxicity studies of the delousing chemotherapeutants on species that are ecologically relevant for the salmon producing nations. The aim of the study was to assess the acute toxicity of H<sub>2</sub>O<sub>2</sub> to three important marine crustaceans of the Norwegian coast: the copepods *Calanus* spp., the Northern krill (*Meganyctiphanes norvegica*), and the European lobster (*Homarus gammarus*).

Though H<sub>2</sub>O<sub>2</sub> has long been labeled as the most environmentally friendly topical treatment for treating sea lice infestations on salmonid farms, this study has shown that recommended H<sub>2</sub>O<sub>2</sub> concentrations are lethal to nontarget crustaceans. Our results demonstrate that H<sub>2</sub>O<sub>2</sub> was acutely toxic *Calanus* spp., *H. gammarus* and *M. norvegica*. We observed that lethal effects occurred

at concentrations well below recommended treatment concentrations. We have identified the keystone krill species, *M. norvegica*, as the most sensitive crustacean to H<sub>2</sub>O<sub>2</sub> of tested so far. Sublethal effects were observed immediately after the 1 h exposure. Our behavioral results suggest that *Calanus* spp., the dominant component of the North Atlantic zooplankton communities, will be more susceptible to predation due to impairment of their escape response as a result of the paralysis induced by H<sub>2</sub>O<sub>2</sub> at concentrations equivalent to 1% of the recommended treatment. And the behavior parameters linked with the shelter seeking behavior of the *H. gammarus* juveniles were negatively affected at concentrations low as 5% of the recommended treatment. The survival of newly settled lobsters depends on the ability to avoid predators and rapidly find a shelter. Young lobsters that reside in the vicinity of salmon farms treating with H<sub>2</sub>O<sub>2</sub>, may therefore be at a higher risk of predation.

#### **2.01.T-03 Transcriptomic Comparison of Lab and Field Based Enchytraeids for Toxicity Testing**

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Organisms are exposed to a wide range of toxicants in the environment and due to the number of species it is impossible to perform chemical risk assessments for all, so model organisms are used. Given the array of toxicants, high-throughput systems using lab cultured species are needed. Many laboratory test species are highly inbred, so may differ genetically to their wild counterparts, so it is important to compare their responses to wild animals.

Annelids fill vital ecosystem functions so earthworms are a key OECD test organism. Enchytraeids can provide similar data over a much shorter timespan. Unlike earthworms, where a realistic population can be field collected, enchytraeids for toxicity testing are reared in artificial culture conditions, hence responses may be very different. Are results from lab experiments due to transcriptomally “lazy worms”?

The effect of environment was compared using transcriptomes prepared from worms taken directly from 1) artificial culture, 2) after exposure in aquatic media, 3) having acclimated for 2 weeks in soil. Very minor differences were seen in the expression of genes relevant to toxicological response

While rearing or exposure conditions may not affect transcriptional profiles of worms, are these cultures representative of the wider population?

A transcriptome atlas has been generated of field collected *E. albidus* and the expression of toxicologically pertinent genes and the expression patterns are being compared with closely related (99% across CO1 genes) individuals from inbred cultures. Given the transcriptomic validation, high throughput aquatic testing was used for 12 commonly used agrochemicals in *E. crypticus*. The transcriptomic responses to a selection of these chemicals (and their mixtures) are being analysed across a highly resolved time-series, revealing mechanistic insights into toxicity.

Overall, rearing conditions do not appear to have a major impact on the transcriptomic output of *E. crypticus*. Wild *E. albidus* are transcriptomally similar to culture for toxicologically pertinent genes. This means inbred lab cultures of enchytraeids, amenable to high-throughput exposure systems, are a good model for soil dwelling annelids. Our research suggests combining these systems and transcriptomics will allow a mechanistic understanding to help predict agrochemical sensitivity across annelids.

#### **2.01.T-04 The Effects of Dexmedetomidine on the Behaviour of Crustaceans: Lab to Field Approach**

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Dexmedetomidine is an  $\alpha_2$ -adrenergic receptor agonist and was originally authorised as a human/veterinary sedative but is now an active ingredient in antifouling paint for boats. In crustaceans it is known to bind to octopamine receptors, which like other neurohormones, cascade to control many biological functions including behaviour. The aim of this study was to determine whether exposure to Dexmedetomidine can alter the behaviour of marine amphipods (*Echinogammarus marinus*) and juvenile crabs (*Carcinus maenas*) under laboratory conditions using both Zantiks and Daniovision behavioural analysis. Amphipod specimens were exposed between 0.1 and 100  $\mu$ g/L with behaviour recorded over several (1-3) days and (1-2) weeks and compared to controls and octopamine exposed individuals. Exposure significantly increased activity vs controls in both amphipods and crabs interesting non-monotonic concentration effects observed. We then set out to determine whether these results could be replicated in the field using acoustic telemetry in large adult *C. maenas* were exposed to Dexmedetomidine and octopamine and tagged and released into a saline lake. The field experiment is ongoing but the results of which will be presented representing the first acoustic telemetry project with crabs looking into the behavioural effects of an environmental contaminant.

#### **2.01.P Advantages of Using Lab and Field Collected Invertebrates and in Situ Studies in Ecotoxicology: Challenges and Opportunities**

##### **2.01.P-Th102 The Amphipod *Gammarus pulex* Thrives in Polluted Habitats but Genetic Diversity is Affected**

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The freshwater amphipod *Gammarus pulex* Linnaeus, 1758 occurs in both pristine and anthropogenically polluted sections of European rivers. Applying population genetics approaches, we addressed the question, whether *G. pulex* is impacted by a selective effect of ecotoxicologically relevant anthropogenic organic micropollutants (AOM) in polluted field sites. We performed a regional scale study with *G. pulex* sampled at differently polluted sites at six rivers in central Germany. The *G. pulex* genetic population structure was analyzed by genotyping 16 microsatellite markers and by comparisons of individual DNA sequences of a segment of the mitochondrial cytochrome oxidase I (COI) gene. In parallel, AOM contamination levels and their toxicity potentials at the sampling sites were determined by measuring an array of AOM in water and amphipod tissue samples. Genetic data indicated intact gene flow within the rivers; a separation of genetically differently adapted *G. pulex* subpopulations by chemical water contaminants was not indicated. However, there was a clear trend of reduced genetic diversity of *G. pulex* from sites with higher AOM levels. Tissue levels of AOM in *G. pulex* were found to depend on AOM levels at the respective sampling sites, with higher AOM tissue levels and accordingly higher chemical toxicity potentials in individuals from the more polluted sites. As indicated by a lab experiment with the model toxicant imidacloprid toxic susceptibility of *G. pulex* for adverse chemical effects was higher when individuals were from a sampling site with increased AOM water and tissue levels. Although *G. pulex* can be abundant in river stretches with high AOM levels our studies, comprising population genetics, chemical analysis and toxicological approaches, show that *G. pulex* is affected by AOM: 1) Continuous exposure to certain AOM levels in the species' habitat leads to an increase of its toxicological sensitivity on the individual scale; 2) AOM caused reduced genetic diversity on the population scale. Reduced genetic diversity and increased sensitivity may lead to a reduction of species resilience to further stressors in its habitat.

## 2.01.P-Th103 Effects of Artificial Light at Night on Two Freshwater Invertebrates

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Light pollution is one of the emerging environmental concerns. ALAN has been expanding worldwide, and it is expected to increase continuously, in the upcoming years, according to IUCN. Artificial light at night (ALAN) leads to changes in natural light timing, intensity, and spectrum. Consequently, human health and biodiversity have been threatened since the day/night light cycle plays a crucial role in living organisms' behavioural and physiological processes. In fact, ALAN, namely from white LEDs that emit a wavelength peak in the blue range, is associated with the suppression of nocturnal melatonin production. Melatonin, the time-keeping hormone, regulates biological rhythms and an array of physiological processes and has been appointed as a key sensitive response to ALAN in many organisms. We present work aimed to assess the effects of ALAN on the life history traits of two freshwater invertebrates: the insect *Chironomus riparius* and the planarian *Girardia tigrina*. This work also aimed to understand the potential role of the hormone melatonin as mediator of light pollution effects.

For that, *C. riparius* were exposed for 28 d to three light regimes (control, i.e., dark nights) and two relevant levels of ALAN (1, 10 lux during the night period), in the absence and presence of exogenous melatonin (1 µM). Larval growth, development time, and imagoes body weight were analysed. *G. tigrina* were exposed for 10 days to the same treatments. After exposure, behavioural endpoints (feeding and locomotion) and cephalic regeneration after decapitation (blastema length, photoreceptors, and auricle formation) were evaluated.

Results show that exposure to ALAN led to delayed emergence of *C. riparius* (significantly for females) and altered males imagoes size. Concerning *G. tigrina*, exposure to ALAN caused a delay in head regeneration, with no clear effects in behaviour. Results also indicate that exogenous melatonin could help mitigate the ALAN-elicited effects suggesting that ALAN might affect its production in aquatic invertebrates. However, further studies are needed to clarify this and ongoing experimental work is evaluating biochemical responses (e.g., energy metabolism and oxidative stress/damage). Results suggest that low, environmentally relevant intensities of ALAN could affect the life history traits of freshwater invertebrates, potentially leading to adverse population-level effects.

## 2.01.P-Th104 Broadening the Perspective of Environmental Stress in Aquatic Ecosystems due to Contamination: An Approach using the Habitat Selection Response Based on a Cost-Benefits Balance

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If it is assumed that mobile organisms move to look for food, a safe habitat, to mate or to avoid predators, why is the same not expected to occur when they are confronted by contamination? What would happen if we looked at the effects of contamination from another perspective, by broadening the way that toxicity is assessed? From a wider conceptual perspective, some particularities that can condition the responses of organisms to contamination should be considered: (i) are organisms able to escape from contaminants thus avoiding their toxic effects? (ii) what is the role of contamination in organisms' habitat selection processes? The aim of this work is to show, firstly, evidences of the role of contaminants in the organisms' decision to stay in or avoid an ecosystem and, secondly, how a nonforced approach could be integrated to the forced exposure approach to understand the balance of cost-benefits performed by organisms when a contaminated habitat is selected. In addition, we will show the different nonforced multicompartimented exposure systems that have been developed at different spatial scales (mini- and

mesocosms) to simulate spatially connected heterogeneous contamination scenarios (methodological advance). Our recent data show that contamination by the agrochemicals chlorpyrifos and terbuthylazine at 10 µg/L was not very aversive to *Daphnia magna*; however, in mixture they considerably prevented the colonization response (even at 0.01 µg/L). In a cost-benefits study, copper showed to be aversive to the freshwater shrimp *Atyaephyra desmarestii*, but when simultaneously confronted to contamination, risk of predation, and presence of shelters, shrimps made an environmental analysis moving toward moderately contaminated area with shelter and lower risk of predation, instead of selecting clean areas with predation risk. Finally, we observed the attraction exerted by fluoxetine on *D. magna* (organisms moved to 800 µg/L) even at lethal concentrations (LC50 was 365 µg/L), disturbing the cost-benefits balance. This approach represents an effort to broaden the ecology component within ecotoxicology, where contaminants are integrated as an ecological element (Stress Ecology), providing a tool that could be used complementarily with the traditional forced exposure approach. Finally, the nonforced exposure approach allows to include ecological concepts as habitat selection and colonization (conceptual advance) to Ecotoxicology.

#### **2.01.P-Th105 Application of a Feeding Inhibition Test with the Mayfly *Cloeon dipterum***

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In recent years, the interest of testing nonstandard species for environmental risk assessment of pesticides has increased. Particularly, interest in mayflies increased due to their relevance in aquatic ecosystems and sensitive response to exposure to certain pesticides.

In this context, we developed a semistatic test system to investigate the food uptake and thus the potential feeding inhibition on the mayfly *Cloeon dipterum*, exposed to different concentrations and exposure scenarios of two insecticides. The exposure scenarios differed in the duration of the preexposure to the test substance (48 h, 24 h, 8 h) which allows the quantification of onset of effects. During the feeding period, the larvae were provided with laboratory grown periphyton. For the periphyton culture, a natural inoculum collected in field was used and the periphyton was grown on unglazed tiles under controlled conditions. The quantity of the periphyton was measured fluorometrically as chlorophyll-*a* concentration at the start and the end of the 96 h feeding period. The experiments consisted of 20 replicates for each of the six concentrations and the control. Each replicate contained one single medium-sized larvae of *C. dipterum* grazing on one tile covered with a predefined periphyton layer. Additionally, five replicates were prepared for each concentration and the control to determine the growth of the periphyton in the absence of larval feeding. The food uptake was calculated as amount of periphyton consumed by a larva over the feeding period corrected by the growth of periphyton without grazing. Despite considerable variability, the results revealed a concentration- and time-dependent decrease in food uptake: effects in lower concentrations tend to increase with the exposure duration also in the absence of signs of immobilization. Nevertheless, further development regarding the standardization and culture of food is needed to particularly reduce variability as observed for the measured endpoints.

#### **2.01.P-Th106 All the Way up? – Does Contaminated Biofilm Affect its Grazer *Cloeon dipterum*?**

**Sophie Oster, Mugilvannan Sivagnanam and Mirco Bundschuh, iES Landau, University of Kaiserslautern-Landau (RPTU), Germany**

Ecosystems are multibranch and multilevelled networks. Aquatic ecosystems are based on multiple connections within and among trophic levels supporting ecosystem integrity. Chemical stress can disrupt these interactions and lead to shifts in structure and function. Direct effects of chemicals have been widely studied, but studies of potential indirect effects are scarcer. Against this background, aquatic biofilms acting both as primary producers and food source for primary consumers were offered as food to the mayfly larvae *Cloeon dipterum* after exposure to chemical stress. To do so, we collected biofilm from a small river in a natural reserve in Rhineland-Palatinate (Germany) serving as inoculum colonising ceramic tiles in the Landau Laboratory Stream Microcosms. After a four-week period of precolonisation, biofilms were chronically (14 days) exposed to 1) 10 µg/L of the herbicide propyzamide, 2) 10 µg/L of the antibiotic ciprofloxacin and 3) to the mixture of both. Subsequently, we fed the biofilm to field collected *Cloeon dipterum* larvae (larval stage 1-3) under controlled conditions for 21 days. Grazers were analysed regarding growth, physiological condition (fatty acid composition), and feeding activity, while the biofilm was analysed in terms of biomass using ash free dry weight data, chlorophyll content, and fatty acid composition. The data are currently being analysed supporting a coherent interpretation of potential bottom-up effects among trophic levels.

#### **2.01.P-Th107 The Use of *Potamopyrgus antipodarum* Feeding Rates as a Sensitive Ecotoxicological Endpoint: Effect of Salinity and Niclosamide**

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*Potamopyrgus antipodarum* is a detritivore freshwater snail species native to New Zealand that has become a successful invader in a variety of freshwater environment. Characteristics such as its worldwide distribution and a wide tolerance to a range of environmental factors led this species to be proposed as a potential model species in ecotoxicology. Additionally, its exclusively parthenogenic reproduction outside its native distribution allows for the easy isolation of clonal lineages. A standard protocol has already been developed for the use of *P. antipodarum* reproductive output (OECD guideline no. 242) as an endpoint to assess potential effects of contaminants exposure.

In this work, we explored the potential use of feeding rate as an additional endpoint in ecotoxicological assays using *P. antipodarum*. We carried out 8 to 10 day feeding tests, in which we measured the leaf consumption (alder leaves discs) of *P. antipodarum* exposed to sublethal levels of salinity and the molluscicide Niclosamide. Results demonstrated the high sensitivity of *P. antipodarum* feeding rates, since significant feeding inhibition was observed in snails exposed to both stressors. These results were also comparable to those obtained with previously performed standard 28-day reproduction tests, in which the number of embryos present inside the oviduct of each female were counted, in response to the same stressors.

The developed bioassay was proven to be as sensitive as reproduction (number of embryos produced per female) presents several advantages in relation to the 28-day reproduction test (since it does not involve the dissection of all test organisms, less time consuming) and offers possibilities to be applied in *in situ* exposures.

## **2.01.P-Th108 Standard Laboratory and Field Experiments to Assess the Toxicity of Three Pesticide Formulations to Collembola**

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Field studies may help to clarify how pesticides change population dynamics and community structures in realistic scenarios. However, ecotoxicity data for most pesticides are only available from single-species tests. The aim of this work is to assess the effects of three commercial pesticide formulations on the diversity of Collembola in an agricultural field in parallel with standard laboratory toxicity tests.

The commercial formulations tested were fungicidal product (200 g/L pyraclostrobin), insecticidal product (100 g/L zeta-cypermethrin), and herbicidal product (300 g/L clopyralid). The toxicity tests were performed following OECD guideline 232 for assessing effects on the reproduction and survival of *Folsomia candida*. The tests used a range of seven concentrations covering the predicted environmental concentrations of the active substances in Lufa 2.2 soil.

Dose-response curves were fit to the data to estimate effect concentrations.

In the field experiment, the formulations were applied at 1× and 10× the recommended dose. Each treatment was replicated in three different plots, randomly placed on the field, and each replicate was sampled three times at each time point. Samples were collected 1 day before pesticide application and 7 and 28 days after application. The number of Collembola was counted, and the diversity of families and species was estimated using Shannon's diversity index. The effects of the pesticides, dose and time were tested using linear mixed-effect models.

The toxicity tests showed that the herbicide and the insecticide had small to no effects on survival, while the fungicide resulted in a LC<sub>50</sub> of 19.6 (95% CI: 17.5 - 21.8) mg a.s. kg<sup>-1</sup> soil. The reproduction of *F. candida* was likewise more sensitive to the fungicide, with an EC<sub>50</sub> of 10.7 (9.4 - 11.9) mg a.s. kg<sup>-1</sup> soil, followed by the insecticide and the herbicide. These values are 10 times higher than the PEC for this active substance after application of 10x the recommended dose. The preliminary results from the field sampling show no significant (p<0.05) effects of chemical dose or time on the abundance and diversity of Collembola. Despite that, the average diversity index of the plots treated with the fungicide was consistently lower than the control on days 7 and 28 after pesticide application. These data will be further analyzed using a trait-based approach to gain more insight into the impact of these pesticides on microarthropod functional groups and soil processes.

## **2.01.P-Th109 Indirect Effects of Antibiotics and Fungicides on Aquatic Macroinvertebrate Food Webs via Bottom-up Regulation**

**Frederik Meyer**, Ralf Schulz and Mirco Bundschuh, iES Landau, Institute for Environmental Sciences, University of Kaiserslautern-Landau (RPTU), Germany

Organic matter of terrestrial origin, such as leaves, represents a large proportion of the energy budget in streams with a forested catchment. Nutrients and energy bound in leaf litter are made accessible to the wider aquatic food web by microbial decomposers (bacterial communities and fungi) and shredding macroinvertebrates. Microorganisms can improve the nutritious quality of leaves for shredders by reducing leaf toughness and by concentrating essential elements (=conditioning). Antimicrobial substances, which are released into streams via wastewater or runoff, can modify the conditioning processes leading to bottom-up directed effects on shredding macroinvertebrates. However, consequences for species interactions translating to changes in the aquatic food web are unclear. Therefore, the main objective of this study was to investigate bottom-up directed effects by antimicrobial stressors in aquatic macroinvertebrate food webs. For this purpose, leaves were conditioned two weeks under exposure to 8 concentrations of an antibiotic (ciprofloxacin) and a fungicide (azoxystrobin), respectively, presuming alterations in the leaf-associated microbial community. After conditioning, these leaves were provided to an extended macroinvertebrate food web in model stream ecosystems (16 x 3.6 m long flow through stream microcosm). During this 4-month experiment several samples were taken. Conditioned leaves were sampled bi-weekly to assess the community of bacteria and aquatic hyphomycetes via next generation sequencing (NGS) and qPCR. At the termination of the experiment for each stream 3 locations were sampled with a self-made Surber sampler (25x25cm) to determine potential community-level effects. Qualitative samples were taken between the three sampling sites for stable isotope and fatty acid analysis. The results are expected to provide new insights into the effect sizes

of bottom-up directed effects in heterotrophic food webs on community structure, trophic interactions and energy reserves. Thereby fundamental knowledge is generated for a field which received little to no attention in the past.

#### **2.01.P-Th110 Sensitivity Differences to Imidacloprid Among Aquatic Invertebrates Can be Explained by Toxicokinetic and Toxicodynamic Models**

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In ecological risk assessment, both standard and nonstandard aquatic invertebrate species are used to increase environmental realism. *Daphnia* is one of the most commonly used standard species and is easily cultured in the lab. *Daphnia* has been used for various chemical effect assessments; however, it could not capture the effect of neonicotinoids, frequently used pesticides. Therefore, a previous study proposes to replace or add additional nonstandard test species, such as insects and crustaceans. In addition, such nonstandard aquatic species are often not lab-cultured but collected from the field before actual testing, which introduces potential uncertainties. For example, researchers often pragmatically select test organisms of specific sex and/or size, which may not represent the sensitivity of the whole population.

To understand the mechanism of these sensitivity differences, effect models like toxicokinetics–toxicodynamics models can be used. Also, lately, these models have been recommended to use in risk assessment for the risk assessment of pesticides for aquatic organisms.

To understand the high tolerance of *Daphnia*, we compared the toxicokinetics and toxicodynamic difference between *Daphnia magna* and several aquatic invertebrates, like Mayflies, Amphipods and Isopods. We found that the high tolerance of *Daphnia* could be explained by its fast elimination rate and lack of the biotransformation of the imidacloprid to a toxic metabolite.

In addition, to compare the intra-sensitivity difference, such as size and sex, we performed acute toxicity and simple toxicokinetic experiments with different size and sex groups (neonates of unknown sex, juveniles of unknown sex, adult females and adult males) for both crustacean species (*Gammarus pulex* and *Asellus aquaticus*). For both species, we found that neonates were the most sensitive group. For *G. pulex*, the sensitivity decreased linearly with size, which can be explained by the size-related uptake rate constant in the toxicokinetic process and size-related threshold value in the toxicodynamic process. For *A. aquaticus*, female adults were least sensitive to imidacloprid, which could be explained by the low internal biotransformation of imidacloprid to a toxic metabolite, imidacloprid-olefin.

#### **2.01.P-Th111 In vitro Bioaccumulation Assessment of Nanoplastics to Freshwater Mussels Hemocytes - Can These Circulating Cells Serve as Proxy of Plastic Pollution in Environmental Biomonitoring Programs?**

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Bioaccumulation is a key process (or factor) in/for the assessment of nanoplastics risk to the environment. Within aquatic ecosystems, freshwater mussels are particularly relevant to serve as bioindicators of plastic pollution due to their high filtration rate and their ability to bioaccumulate and bioconcentrate toxicants. However, methodologies supporting nanoplastics bioaccumulation assessment, facilitating the understanding and data integration into comprehensive biomonitoring programs using these organisms are lacking. Moreover, their suitability for inter-species comparisons, *in vitro-in vivo* extrapolation and the lack of accurate predictive procedures remain major challenges.

Owing to an open circulatory system, freshwater mussels hemocytes may constitute one appropriate noninvasive *in vitro* alternative for a mechanistic understanding of the nanoplastics associated effects. This approach could then be applied on environmental biomonitoring campaigns that will permit to prioritize and survey nanoplastic contaminated areas, prevent harmful exposures to the public and evaluating policy interventions related to environment plastic exposure risk mitigation and management.

In this work, freshwater mussels were used to investigate hemocytes aptness to act as swiftly and early sentinel of nanoplastic exposure. Hemolymph of mussels was (noninvasively) extracted from the posterior adductor muscle and hemocytes were isolated from plasma, counted and cultured to conduct functional *in vitro* assays relevant for nanoplastics bioaccumulation assessment.

#### **2.01.P-Th112 Performance and Robustness of the ex situ Continuous Biomonitoring System for Coastal Waters Based on a Heart Rate of Mediterranean Mussel (Mytilus Galloprovincialis Lamarck, 1819)**

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The focus of this research was the assessment of the robustness of *ex situ* biomonitoring system based on physiological biomarker, namely heart rate of Mediterranean mussel. Experimental set-up provided not only insight into reliability of the early warning system and variability of heart rate over a year-long continuous operation but enabled defining the optimal shelf life of bioindicator organism in the continuous biomonitoring. Basal heart rate dynamics in control conditions, with no significant stress,

demonstrated considerable time-dependent individual and group variability. Still, the responses of all tested specimens, regardless of their individual variability, followed a similar behavioral trend, which implies that the test individuals can be clearly regarded as a group. The responses of selected physiological biomarker did not follow circadian or seasonal pattern. The sensitivity of automated *ex situ* system and selected species to natural variation of a number of environmental variables has also been investigated. The results indicate that the mussels' heart rate is not affected by the key environmental factors provided they remain within the usual variation range typical for the selected site. The overall physiological condition of test individuals and performances of monitoring system have been tested on monthly basis by group response to short-term exposure to extreme stress conditions (a sharp salinity decrease). Selected biomarkers, coefficient of variation, and recovery time of heart rate after the exposure to stress proved to be good indicators of condition of individuals in monitoring system. Successive increase of the heart rate recovery time was recorded already from the second month of monitoring onwards, while the complete absence of an expected response to stress – change of the heart rhythm – was apparent during the last four months of monitoring. These results indicate that the individuals in the monitoring system have limited optimal shelf life and that after three to four months of continuous monitoring, the system is subject to "fatigue". To maintain the optimal performance of the system, individuals need to be gradually replaced after three to four months.

#### **2.01.P-Th113 The Effects of Plastic Additives on Male Fertility and Reproductive Behaviour in Marine Amphipod**

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In a plastic-dominated world, there are predicted to be over 10 000 chemical additives, of which over a quarter are reported to be neurotoxic, immunotoxic, and endocrine disruptors. Most are not chemically bound and are commonly known to leach out into the environment. The ban on legacy pollutants like di-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) does not preclude their potential toxic effects on already exposed biota. At the same time, other groups of plastic additives, including n-butylbenzenesulfonamide (NBBS) and triphenyl phosphate (TPhP), have now been placed on the international watchlist for evaluation. This study aims to assess the effects of these plastic additives on male fertility and reproductive behaviour in the marine amphipod, *Echinogammarus marinus*. We assessed male infertility by exposing male amphipods to environmental and laboratory concentrations of DEHP, DBP, TPhP and NBBS for 14 days, after which sperms were counted. For reproductive behaviour, 20 precopulatory pairs of *E. marinus* were exposed to varying concentrations of the test compounds. High throughput optimization of the precopulatory pairing behaviour was developed and repairing success was recorded at 15mins and daily for 96 h. Sperm count declined after 14 days of exposure. Animals exposed to DBP and TPhP showed a dose-response relationship with significant differences in the highest concentration. Repairing success was impacted by the presence of the test compounds even at low concentrations. Animals in the exposed group took a long time to reform pairs. The percentage of repairing success ranged from 80 – 100 % in the control group and 0 – 40% in the highest concentration for DEHP, DBP, NBBS and TPhP. Overall, this study provides evidence that reproductive mechanisms and sperm parameters of amphipods can be impacted by plastic additives even at environmentally relevant concentrations. Thus, demonstrating the potential of this endpoint in toxicity testing and its implications on population-level effects.

#### **2.01.P-Th114 Assessment of the Toxic Effects of 10 Types of Detergents on the Microcrustacean *Hyalella azteca***

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The detergents are synthetic compounds, containing surfactants and additives. They alter the water quality and are toxic to aquatic organisms. Due to the fact that in our country the studies of these products are scarce, in this work the toxicity of 10 commercial brands of detergents, which are used for washing dishes, washing clothes, washing hands, and general use, in the microcrustacean *Hyalella azteca*, was evaluated. Bioassays lasting 96 h were performed. Twenty organisms were exposed to 7 detergent concentrations (1, 5, 10, 20, 40, 60, and 80 mg/L) in triplicate, plus a nontoxic control. With the mortality data, the LC<sub>50</sub> was determined. Subsequently, a bioassay was carried out exposing the microcrustaceans to 3 sublethal concentrations. After 4 days of exposure, the level of lipid peroxidation (TBARs) and the activity of the AchE enzyme were determined. In the data obtained, significant differences were observed between the response of the control group and the organisms exposed to the detergents ( $p < 0.05$ ). The most toxic detergent was a product used for washing dishes and the least toxic was a product for general use. The highest TBARs levels were recorded in organisms exposed to detergents used for dishwashing and laundry (from 76.5 to 52.4 nM TBARs/mg) and the lowest in microcrustaceans exposed to a general use product (10.6 nM TBARs/mg) (the value obtained in the control was 3.1 nM TBARs/mg). A decrease in AchE activity of between 8 and 46% was observed in organisms exposed to dishwashing, handwashing, and laundry detergents. Since wastewater treatment is limited and detergents are discharged directly into the environment, it is important to know the potentially adverse effects of these compounds in order to propose mitigation measures.

#### **2.01.P-Th115 Effects of Exposure to Cadmium on Juveniles of Pearl Oyster *Pteria sterna* (Gould, 1851)**

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*Pteria sterna* is an economically important bivalve because it produces high-quality pearls. Currently the natural populations of these organisms have disappeared; the cause is attributed only to overexploitation, since there are no environmental studies to

detect other possible variables that cause the disappearance of the banks. Due to the lack of studies on the effect of metals on *Pteria sterna*, the objective of this work is to evaluate the toxic effect of cadmium on pearl oyster juveniles. Bioassays lasting 48 h were performed where 5 metal concentrations were tested (5, 1, 0.5, 0.1, 0.05 mg L<sup>-1</sup>). With the mortality data, the LC<sub>50</sub> was calculated. The bioassays evaluated: respiration rate, excretion rate, O:N ratio, lipid peroxidation degree (Tbars) (in gills) and acetylcholinesterase (AChE) enzyme activity (in foot tissue). The LC<sub>50</sub> obtained was 1.52 mg L<sup>-1</sup> (48 hours). Significant differences were observed between control and exposed organisms ( $p < 0.05$ ). The respiration rate of oysters exposed to cadmium was 40% to 80% lower compared to the control. The excretion rate increased between 20% to 60%. Cadmium had an inhibitory effect between 18 and 72% on the activity of the acetylcholinesterase enzyme. The degree of lipid peroxidation in oyster tissues increased up to 200%. The O:N index had values below 10, indicating a high degree of stress. According to our results, it was evident that cadmium in sublethal concentrations has a deleterious effect on oysters, increases the degree of stress, and has an oxidative and neurotoxic effect.

## **2.01.P-Th116 Comparison of the Acute Toxicity for Two Pyrethroid Pesticides to *Chironomus riparius* and *Glyptotendipes tokunagai***

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The chironomid is a benthic organism and affected by pesticides adsorbed to sediments or remained in sediments through elution into water systems and river flooding. Pyrethroids are synthetic insecticides with chemical structures similar to those of natural insecticides, pyrethrins, and are used worldwide. Etofenprox and fenpropathrin are pyrethroid pesticides, known to have the log P value higher than 3. It means there is a high possibility of soil adsorption. Currently, environmental risk assessment is conducted for aquatic (fish, water fleas, and green algae) and terrestrial organisms (honey bees, earthworms, and birds) for pesticide registration in Korea. However, since benthic organisms are not included in the evaluation target, it is necessary to expand the evaluation species reflecting various habitats. Therefore, we compared the toxicity of *Chironomus riparius* and *Glyptotendipes tokunagai* (the Korean native species) to etofenprox and fenpropathrin to set up the acute toxicity test and risk assessment criteria for benthic organisms. The result of the acute chironomid toxicity tests showed that the 48h EC<sub>50</sub> values of *Chironomus riparius* and *Glyptotendipes tokunagai* for etofenprox were 0.00112 and 0.00691 mg/L, respectively. In addition, the 48h EC<sub>50</sub> values of fenpropathrin for *Chironomus riparius* and *Glyptotendipes tokunagai* were 0.0000472 and 0.00600 mg/L, respectively. *Chironomus riparius* showed a higher toxicity sensitivity to etofenprox and fenpropathrin than *Glyptotendipes tokunagai*.

## **2.02 Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment**

### **2.02.T-01 Novel Genomic Markers of Susceptibility to Paraquat, Diuron, and Atrazine in *Chlamydomonas reinhardtii***

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Compared to other environmentally relevant chemicals, herbicides are among the best researched. For most herbicides, there is information not only on the direct molecular targets, but also on the downstream events that eventually lead to weed death, and sometimes on the molecular mechanisms of herbicide tolerance in different plant and algal species.

In this study, we wanted to see whether functional genomics performed with a genome-wide mutant library could provide new information on the susceptibility of the unicellular green alga *Chlamydomonas reinhardtii* to three of the most commonly studied herbicides: diuron, atrazine, and paraquat. Diuron and atrazine are herbicides/algicides that bind to the D1 protein of the PSII reaction center (but at different sites), blocking ATP synthesis, while paraquat disrupts the electron flow in PSI and destroys plant tissue by generating free radicals that attack plant lipid membranes. Our hypothesis was that the mutant screening would provide supporting evidence for the known mechanisms of action of the herbicides, but would also shed new light on their exact mechanisms of action.

For diuron and atrazine, there was some overlap between the genetic markers discovered, with most markers related to photosynthesis: Mutants of Cgl54 (PSII D1 precursor processing protein) were more sensitive to both herbicides, while the homolog of Lpa2 (PSII accumulation protein) was more sensitive to diuron alone and Cre11.467652 (component of psaA trans-splicing sub complex I) was more sensitive to atrazine. For paraquat, several mutants were more tolerant to the herbicide, including a putative paraquat transporter and several genes involved in the synthesis of very long fatty acids and ceramides. Several PSI associated mutant we more sensitive (e.g., the recently PSI associated kinase Cpl3). The length-specific modification of ceramides has been linked to the neurotoxic effects of paraquat in small mammals but not in plants/algae, thus representing a potentially novel mechanism of action for paraquat in green algae.

Based on functional genomics as well as previous knowledge, we have developed a new putative adverse outcome pathway for green algae that begins with chloroplast production of ROS and leads to cell death.

### **2.02.T-02 Exposure of *Lemna Minor* (Common Duckweed) to Uranium and Perfluorooctanoic Acid (PFOA) and Associated Biological Effects**

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A variety of processes, both natural and anthropogenic, can have a negative impact on surface waters, which in turn can be detrimental to human and environmental health. Few studies have considered the ecotoxicological impacts of concurrently occurring contaminants, and that is particularly true for mixtures that include contaminants of emerging concern (CEC). In response to this knowledge gap, our study considers potential biological effects in *Lemna minor* (common duckweed) induced by a ubiquitous radionuclide (uranium, U) and a fluorinated organic compound (perfluorooctanoic acid, PFOA, considered a CEC) alone and in combination under controlled laboratory conditions. *L. minor* was grown for 5 days in small, aerated containers. Treatments were: 0, 0.3, and 3 ppb PFOA; 0, 0.5, and 5 ppb U; and combinations of these. Each treatment consisted of four replicates with 7 plants each. Plants were observed daily for frond number and signs of chlorosis and necrosis. Other biological endpoints examined at the conclusion of the experiment were average specific growth rate, chlorophyll content, and antioxidant capacity. Analysis of variance and Tukey's pairwise comparisons were used to determine the significance of the effects of relevant factors on biological endpoints of interest. In single exposure experiments, both PFOA and U concentration significantly impacted frond number ( $p = 0.0005$  and  $p < 0.0001$  respectively), with the suggestion of a stimulatory effect at 0.3 ppb PFOA and inhibitory effect at 0.5 and 5 ppb U. In the dual exposure experiment, inhibitory effects on frond number were also observed in the contaminant mixtures with high U concentration. PFOA treatment did not significantly influence the average specific growth rate ( $p = 0.1206$ ), but the U treatment did ( $p = 0.0015$ ). Treatment was also a significant contributor to variation in specific growth rate in the dual exposure experiment ( $p < 0.0001$ ). Taken together, these results suggest that uranium has a more dominant effect on effects observed in *L. minor* than PFOA.

## 2.02.T-03 Can we Predict Regime Shifts in Shallow Aquatic Systems Exposed to Multiple Stressors? – Conclusions from Micro- and Mesocosm Experiments

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Shallow aquatic systems are abundant in agricultural landscapes but highly susceptible to suffer from climate warming and agricultural run-off (ARO). Regime shifts between the dominance of microalgae or macrophytes may occur depending on the level and interaction of multiple stressors acting on the system. The main objectives of our French-German project CLIMSHIFT were (I) to understand the response of shallow aquatic ecosystems to warming and ARO based on a mechanistic understanding of stressor interactions and community responses; (II) to evaluate if the stressors act in an additive, synergistic or antagonistic way on organism physiology, community and ecosystem processes; and (III) to determine potential thresholds for shifts in ecosystem functions for single and combined stressors. CLIMSHIFT included a series of indoor microcosm experiments testing different exposure scenarios to ARO combined with warming on a benthic-pelagic community typical for shallow aquatic systems. Those were upscaled to larger outdoor mesocosms using natural plankton and periphyton communities. In all micro- or mesocosm experiments, we observed regime shifts from the good ecological status with a dominance by macrophytes to a degraded status with a dominance by either phytoplankton, periphyton or blanketing algae. Our CLIMSHIFT project proved that a well-planned experimental design based on a combination of micro- and mesocosm experiments allows to study complex multiple stressor effects on shallow aquatic systems, and to disentangle direct and indirect effects as well as stressor-interaction patterns. Combined nutrient (nitrate) and pesticide effects led to a range of direct and indirect effects on primary producers and consumers, with the most visible being the shift from macrophyte dominance to the dominance either phytoplankton, periphyton or blanketing filamentous algae. Nitrate and warming acted synergistically with pesticides, leading to stronger macrophyte decline. Further, warming can lower the threshold for such shifts. Effects of agricultural run-off on these systems are especially severe in spring, when average rainfall is high and submerged macrophytes start to grow. Lowering local stressors can help maintain shallow aquatic systems in a good ecological status even when global stressors such as climate warming prevail.

## 2.02.T-04 Use of Visual Phytotoxicity for ER50 Derivation in Nontarget Terrestrial Plant Studies

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Despite the OECD 208 and 227 guidelines indicate that visual phytotoxicity effects should be reported, they do not clearly state the need to derive ER50 based on phytotoxicity. However, in its recurring issues in ecotoxicology document (2019), EFSA stated that “the EC<sub>x</sub> based on phytotoxicity should be reported” and “it should be considered for the Tier 1 risk assessment” in case it is the lowest endpoint. As visual estimates may have several sources of imprecision and bias, the reproducibility of visual phytotoxicity assessments for possible derivation of ER50 values and their use in the European risk assessment of nontarget terrestrial plants (NTTP) has been evaluated in this vegetative vigour study.

Five plant species (onion, oilseed rape, soybean, sugar beet, tomato), typically used in NTTP studies, were sprayed with two herbicides with different modes of action. An untreated control and five treatments (from 0.0625x to 1x of their field use rate) were assessed for each herbicide. Phytotoxicity was recorded 21 DAT and evaluated based on photographs. A total of 1227 photos were made available to 23 assessors, who are familiar with and regularly assess visual phytotoxicity in their job. By comparing to the untreated plants, assessors assigned phytotoxicity scores in a blind fashion to the treatments (except for the control plants) using a 0 to 100% scale.

The agreement in scores recorded by the assessors was determined using the Intraclass Correlation Coefficient (ICC), preliminarily for three of the plant species considered in the study, i.e., tomato, sugar beet, and onion.

The ICC derived for tomato and onion were mainly in the poor range with large confidence intervals, indicating a high variability in the scores assigned by the assessors. A better alignment in the assigned scores could be observed for the high treatment rates of one of the herbicides. In the case of sugar beet, despite some large differences between assessors, ICC were mainly in the range of moderate to good, indicating a better concordance of the scores assigned by the 23 assessors, compared to onion and tomato.

The results from this study shed doubts on the use of ER50 values based on visual phytotoxicity scoring in the European ecotoxicological risk assessment for NTTP because inconsistent risk management decisions may be reached.

## **2.02.T-05 Biochar and Other Amendments for Douglas Fir Survival and Growth on Acidic Mine Tailings**

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In the western US and in other nations, there are many abandoned mine sites with heavy metal contaminated tailings that prevent the reestablishment of vegetation and contaminate local ground and surface waters. Successful growth of plants on these sites is often limited by low tailings pH and high tailings heavy metal concentrations, poor structure, compaction, diminished water holding capacity, and limited fertility, carbon, and microbial activity. In a field study at an abandoned mine, we evaluated the effects of a mixture of amendments (lime, biosolids, biochar, microbial inoculum) on tailings pH and metal concentrations; and subsequent impacts on injury, growth and needle elemental concentrations for Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] over multiple years. Two years post-tree planting, some trees had died, which was associated with lower tailing pH's. However, for the many live trees the tailings had increased pH, lowered metal availability, and increased moisture retention resulting in seedling survival, growth, and adequate needle nutrient contents for growth; while having reduced needle heavy metal concentrations, such as Cu, vs. dead trees. By four years postplanting, tree growth slowed and trees exhibited nutrient deficiency which may be alleviated by additional fertilization. This study highlights the potential for amendments that include biochar, to enhance plant growth by improving soil chemistry and physical conditions. Our amendment recipe includes approximately 2.5% biochar (by weight), along with lime (1%) and biosolids (0.25%), to promote tree survival and growth on mine tailings. This study also points out the necessity for long-term monitoring of plant responses so that, if needed, additional amendments such as biosolids or lime can be added to sustain tree growth.

## **2.02.P Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment**

### **2.02.P-We039 Identifying an Alternative Organic Matter Source for Artificial Sediment Used in Aquatic Plant Exposures**

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Artificial sediment is required for most OECD guideline studies used to evaluate the effects of chemicals on benthic communities, including OECD TG 239, the Water-Sediment *Myriophyllum spicatum* Toxicity Test. It is preferred over natural sediment as it is standardized, which removes the substrate as a variable in the experiments. Peat moss is a component of the guideline artificial sediment mixture, but there has been discussion in recent years that an alternative to the product needs to be considered. Peat moss can only be harvested from delicate wetlands where it provides hydrological services such as water filtration and flooding prevention. Peatlands are also critical carbon sinks, which means that peat extraction has a high carbon footprint. As peat takes hundreds of years to form and the current harvest rate is generally greater than the replacement rate, the impacts from its extraction are difficult to mitigate directly. Another concern is the inconsistency of peat harvested from different regions, as variability in both biological performance and sediment-chemical interactions have been observed; an organic product that can vary widely based on its source is in conflict with the objective of standardization.

A number of scientists have evaluated alternatives to peat moss including coconut coir, wood products, compost, rice hulls, and  $\alpha$ -cellulose that provide a source of organic matter similar to peat. For this project, coconut coir was identified as the most desirable replacement since it is easily accessible and cost-effective and has a similar texture to finely ground peat moss. Coconut coir is a by-product of the coconut industry, and while processing it also has negative environmental implications, it is considered a more responsible option and more consistent in composition when compared to peat. Our first trial compared growth of *M. spicatum* plants grown in two artificial sediments: one made with peat moss and one with coconut coir, and resulted in nearly equal growth between the two test groups. A reference test was then conducted with 3,5-DCP to compare potential differences in toxicity to the reference toxicant between the two sediment groups, and resulted in EC<sub>50</sub> values within historical ranges for both. Additional work will include reference tests with other compounds (e.g., 2,4-D, trifluralin, isoproturon), and similar trials completed with alternative plant species such as *Vallisneria americana* and *Elodea canadensis*.

### **2.02.P-We040 Protectivity Check of the Tier-1 Pesticide Risk Assessment for Aquatic Primary Producers Based on EFSA Endpoints**

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Within the context of Regulation (EC) No 1107/2009 effect assessment procedures may be based on a tiered approach. In Europe, it is usually algae and/or vascular plants that determine the environmental risks in the aquatic Tier-1 effect assessment for plant protection products with an herbicidal mode of action (MoA). This tier includes tests with at least two algae and one macrophyte (*Lemna* sp.). If algae and/or vascular plants are the most sensitive group in Tier-1, higher tiers (e.g., micro/mesocosm studies) should focus on this most sensitive group. In recent years several authors compared the Regulatory Acceptable Concentrations (RACs) from Tier-1 with Tier-3 RACs from micro/mesocosm studies with algae and macrophytes to check the protectivity of Tier-1 risk assessment. These efforts resulted in different conclusions about the protectivity of Tier-1. Therefore, research was undertaken to answer the question “Is the pesticide risk assessment for plant protection products still protective after moving from the  $E_{b/y/r}C_{50}$  to  $E_rC_{50}$ ?” by using the data from the Lists of Endpoints published in EFSA conclusions. For 13 herbicides and 3 fungicides with herbicidal MoA (16 in total) the check on protectiveness could be performed based on Tier-1 and Tier-3 data published by EFSA. When moving from  $E_{b/y/r}C_{50}$  to  $E_rC_{50}$  our results show that in 68 % of the cases (11 substances) protectivity was maintained, as all these values are situated above the line 1:1. This means that in these cases EU Tier-1 is protective for Tier-3. For two substances (12.5 %) a change in protectivity was observed when  $E_rC_{50}$  was used instead of an  $E_{b/y/r}C_{50}$ , as the values drop below the line 1:1. For two substances (12.5 %) both  $E_{b/y/r}C_{50}$  and  $E_rC_{50}$  are below the line 1:1 and Tier-1 is not protective for Tier-3. For one substance (Linuron; 6.25%), no relevant EFSA risk assessment is available thus a conclusion on the protectivity cannot be drawn. When moving from  $E_{b/y/r}C_{50}$  to  $E_rC_{50}$  in conjunction with the standard Assessment Factor of 10, protectivity changes from 81% to 68% of the cases.

## 2.02.P-We041 Effects of the Fungicide Tebuconazole with Herbicidal Mode of Action on Monocot and Dicot Macrophyte Species with Different Growth forms

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Since 2010, it is known that dicot macrophytes, such as *Myriophyllum* sp., can be significantly more sensitive to herbicides with specific mode of actions (MoA) such as auxins than the standard monocot test species *Lemna* sp.. For fungicides such as tebuconazole (TBZ) with a not well-known herbicidal MoA, it is questionable if dicot macrophytes are also more sensitive than the monocot duckweed *Lemna gibba* which was the most sensitive species in acute laboratory tests with TBZ in the first tier of the risk assessment. Therefore, we performed 14 d in-situ macrophyte tests within a comprehensive mesocosm study (project AquaFungi at Risk) at the artificial stream and pond facility (FSA) of the German Environment Agency. The goal of this research was to improve the risk assessment of fungicides with herbicidal MoA for higher aquatic ecosystem/macrophyte protection. To study this, 8 naturally established freshwater mesocosms were dosed once with 6 different concentrations of TBZ (5-5000 µg/L) in the beginning of September 2022. We exposed *Myriophyllum spicatum* shoots in bioassays consisting of 10 plant pots in each mesocosm. Exposure was 14 days with starting dates on day 1 and day 21 after TBZ application, and 35 days, covering the full exposure period for the macrophyte bioassays. Colonies of *Lemna trisulca* and *Spirodela polyrrhiza* were exposed in floating devices for 14 days starting at day 1 and at day 21. Endpoints including main shoot length, total shoot length, number of whorls, total length of side shoots and dry weight were measured for *Myriophyllum spicatum* while dry weight and number of leaves were measured for *L. trisulca* and *S. polyrrhiza*. In addition, floating leaf development of free-growing populations of the dicot species *Nymphoides peltata* and of the monocot species *Potamogeton natans* were measured in the mesocosms. The results indicate that dicot macrophyte species are more sensitive to TBZ than the monocot species. The Risk Assessment of fungicides should therefore include additional macrophyte testing if the fungicide shows herbicidal MoA.

## 2.02.P-We042 Transcriptomic and Proteomic Analysis of Ecotoxic Modes of Action in *Myriophyllum spicatum*

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The increasing use of chemicals such as pharmaceuticals, herbicides and pesticides, as well as their subsequent influx into the environment, pose a constant threat to aquatic ecosystems. The effect on aquatic primary producers is tested and the ecotoxicity of pesticides is, among other species and tests, evaluated using *Myriophyllum spicatum* in the OECD guideline test 239. While these standard ecotoxicological tests enable the regulatory assessment of substances, they are limited to measuring the physiological impact, i.e., growth rate reduction. Despite the importance of mechanistic insights, the mode of action resulting in ecotoxicological effects remains unknown. This study aims at the establishment of a suitable workflow and first application to study molecular effects of toxicity in *M. spicatum*.

Transcriptomic and proteomic profiling are applied to a shortened OECD guideline test 239. That way, molecular effects of two well-studied chemicals – the herbicide bentazon and the pharmaceutical atorvastatin – will be studied using RNA sequencing (transcriptomics) and LC-MS/MS based proteomics. Bentazon is a photosynthesis inhibitor targeting the photosystem II and is commonly used as a postemergence herbicide. The cholesterol lowering atorvastatin targets a key enzyme of the isoprenoid pathway, the 3-hydroxy-3-methylglutaryl-CoA reductase, which is ubiquitous in eukaryotes. Both substances have been recently tested in a similar analysis with the duckweed *Lemna minor*, also commonly used for the evaluation of toxic effects on freshwater plants.

As of now, the plants have been exposed to low effect concentrations, corresponding to the EC<sub>5</sub> and EC<sub>20</sub> values, which were determined in range finding tests. The total RNA and protein of the shoot tips have been extracted and are being analysed. RNA-seq is currently being performed to identify potential molecular effects and suitable biomarkers using a *de novo* assembly. The data will be compared and supported with the proteome derived from a nano-flow LC-MS/MS analysis. It is anticipated that distinct molecular effects can be observed and linked to the known modes of action of bentazon and atorvastatin. As the anticipated molecular changes may possibly be observed before physiological effects occur, the assessment of biomarkers derived from this may potentially be consulted in future studies for early predictions of toxic effects on organisms and aid the development and toxicological assessment of new substances.

## **2.02.P-We043 A Review of Laboratory Cultivation of *Laminaria hyperborea*, *Laminaria digitata*, and *Saccharina latissima* as a Basis for Ecotoxicology Studies**

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*Laminaria hyperborea*, *Laminaria digitata*, and *Saccharina latissima* are dominant kelp species in the South Coast waters of the UK. The two *Laminaria* species provide a foundation for highly biodiverse UK kelp forests. *S. latissima* is an early colonizer of barren habitats that also forms the basis for commercial kelp cultivation. Kelp forest recovery is a critical element in large-scale seascape restoration along the UK South Coast to buffer climate change impacts and address biodiversity loss. Globally, kelp cultivation is also under development as a key component in global climate change mitigation and adaptation strategies. In the UK, there are increasing concerns regarding the impact of suspended sediments and associated contaminants on kelp survival and growth. These concerns from the public, nongovernmental organisations and government authorities are driving demands for kelp ecotoxicology studies to establish the physical, chemical and biological effects of these sediments. Published protocols, and techniques developed in the field, aim to establish viable macroalgal cultures. Yet, diverse protocols, including inducing osmotic stress to stimulate spore release, applying germanium dioxide to kill diatoms, and abrasion to reduce contamination, are recommended even when the biological impact of their use is often unclear and could illicit resultant stress in the studied species. These stressors may increase susceptibility to environmental toxins leading to experimental results not reflecting the organism's natural response. This review examines both the academic literature and 'custom and practice' within kelp restoration projects and commercial ventures to identify undefined stress effects within existing approaches that, unless defined and quantified, will undermine the validity of ecotoxicology studies. Establishing the impact and magnitude of stress effects is essential in developing a kelp ecotoxicology test and associated protocols.

## **2.02.P-We044 Establishment of a Flow-Through System for the Macrophyte Growth Inhibition Test (OECD 239) Including New Endpoints on Photosynthetic Activity**

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Maintaining constant exposure concentrations during ecotoxicological studies while testing rapidly degradable substances is a challenge. Stable concentrations during exposure are often achieved using flow-through systems. The water-sediment toxicity test with *Myriophyllum spicatum* (OECD TG 239) currently only includes a static and semi-static test design. The aim of our study was to establish a flow-through system for *M. spicatum* toxicity testing.

The standard test design was miniaturised from 2 L to 500 mL and a flow-through system was developed to achieve stable exposure concentration of rapidly degrading substances. The main test design and parameters such as light, temperature and pH were kept as described in the OECD TG 239. The observed endpoints were total shoot length (TSL), fresh (FW) and dry weight (DW). As an additional endpoint, measurement of photosynthetic yield (Y(II)) was established to detect early effects on photosynthesis.

The miniaturised system was compared with the standard test setup using 3,5-dichlorophenol. No significant differences could be detected after 14 days exposure. To prevent excessive growth ¼ Smart & Barko medium was tested as a flow-through medium and achieved similar growth to the standard test setup.

Two model substances were chosen to compare their toxicity in the flow-through and in the static/semistatic system. At first, using the PSII inhibitor bentazone (BT), the growth rate of FW and TSL showed 5-8 times higher EC<sub>50</sub> values in the flow-through system but with overlapping confidence intervals and no difference for the EC<sub>50</sub> values of TSL, FW and DW for yield. Using the PAM method with BT, the Y(II) decreased concentration-dependent from day 3. This reduction in Y(II) could be shown to be correlating with the growth rate of the plants. It suggests that the PAM method can detect effects on photosynthesis even before physiological effects can be measured.

Atorvastatin, a pharmaceutical, degraded by 30% after 7 days in a semistatic test design, but remained stable in the flow-through system. Stronger visual effects (e.g., necrosis) were observed, as well as lower EC<sub>20</sub> values for all endpoints, e.g., 1.8 vs. 4.4 mg/L for FW, indicating that the flow-through system is suitable for testing of rapidly degradable substances.

Summed up, the miniaturised and standard tests did not differ, degradable substances can be tested more accurately using a flow-through setup, and photosynthetic yield can be an additional endpoint.

## 2.02.P-We045 Testing Strategy for Species Sensitivity Distribution (SSD) Tests with Aquatic Plants

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The guidelines OECD 238 and 239 with the aquatic plant *Myriophyllum spicatum* were set up to cover toxicity on rooted macrophytes in addition to the OECD guideline 221 which covers free floating species. By now it became obvious that the focus on one submerged rooted and one free floating species might under or overestimate effects based on the mode of action of test items. Therefore, further tests and ring-tests with additional species were set up to refine risk assessment.

In most cases the species sensitivity distribution (SSD) approach with at least eight species is used. This leads to the general questions which species show good growth under lab condition, are relevant for the ecosystem, represent different growth forms, and finally can close missing data links. Also, it is essential to follow the standard test protocols as far as possible to compare data of nonstandard species with standard species. We present data of nonstandard macrophyte test species which fulfilled validity criteria according to OECD 239 and recommend a test strategy based on handling, growth form, and sensitivity of test plants.

## 2.02.P-We046 Assessment of the Harmful Effects of Caffeine on Macrophytes *Lemna gibba* and *Egeria densa*

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Caffeine is one of the most widely consumed stimulant substances worldwide present in food, beverages, and medicines. It is currently considered an emerging pollutant due to its continuous contribution to aquatic systems, where concentrations of 0.007 mg/L to 3.594 mg/L have been detected. Studies on the effects of caffeine on aquatic organisms are scarce. In this work, an evaluation of the harmful effects of caffeine on 2 aquatic plants, the macrophytes: *Egeria densa* and *Lemna gibba*, was carried out on their growth, pigment concentration and their macromolecule content. The macrophytes were exposed for 14 days to five concentrations of caffeine: 0, 0.01, 0.1, 1.0, 10, and 100 mg/L in duplicate. At the end of the exposure period, growth (length and weight) was evaluated to determine the EC<sub>50</sub> (concentration that inhibits the growth of macrophytes by 50%). In addition, the levels of chlorophylls, carotenes, and macromolecules (lipids, carbohydrates, and proteins) were determined. The data obtained showed that there were significant differences in the response of the macrophytes exposed to caffeine and the control group. Caffeine had deleterious effects on macrophytes. The EC<sub>50</sub> obtained in the tests with *Lemna gibba* was 7.4 mg/L and 0.07 mg/L for *Egeria densa*. A decrease in the concentration of chlorophylls that varied from 2 to 68% was observed. In the evaluation of macromolecules, a decrease in carbohydrate concentrations from 20 to 46% and lipids from 15 to 60% was detected. The chlorophyll *a*/chlorophyll *b* ratio indicated that the macrophytes exposed to caffeine were under stress conditions. In sublethal concentrations, caffeine had deleterious effects on the macrophytes *Lemna gibba* and *Egeria densa*.

## 2.02.P-We047 Allometric Relationship of Shoot and Plant Weight in *Myriophyllum spicatum*

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The term allometry originally referred to the scaling relationship between the size of a body part and the size of the body as a whole, as both grow during development. More recently allometry has been expanded to refer to biological scaling relationships in general, be it for morphological, physiological, or ecological traits. It is hence plausible to assume that, for plants, the relationship between total plant weight and shoot weight is an allometric one.

Control values for shoot and whole plant weight were available for 43 *Myriophyllum* studies (conducted to OECD TG 239). Studies included 10 replicates with one plant each per control. Five studies additionally contained solvent controls, which were included in the database, resulting in a total of 474 individual datapoints being evaluated.

Based on the standard allometric equation  $Y = aX^b$  (i.e., *a*: proportionality coefficient and *b*: slope of the regression line), shoot weights (*Y*) were predicted from whole plant weights (*X*) for every datapoint. To this end, the two parameters were log-transformed and then plotted on a log-log plot, from which the allometric function was derived. The *R*<sup>2</sup> of the resulting regression was 0.914.

To test the goodness of the function, predicted shoot weight was compared to the actual shoot weight for any given plant, resulting in a correlation coefficient of 0.956. Additionally, a regression model was run to evaluate the goodness of fit of the model, resulting in an *R*<sup>2</sup> of 0.914. Finally, the average, standard deviation and coefficient of variance (CV) were calculated for both predicted and actual shoot weights based on the whole dataset. Both the average and standard deviations of predicted shoot weights corresponded to those of actual shoot weights, resulting in highly comparable CV (31.9 vs. 31.6%).

The same approaches were then used to calculate the predicted yield based on the predicted shoot weight data, resulting in a correlation coefficient of 0.955 with the actual yield data. The regression model used to evaluate the goodness of fit had an *R*<sup>2</sup> of 0.911, confirming a high concordance between actual and predicted yield values.

In conclusion, a reliable allometric relationship between shoot weight and whole plant weight was derived. This equation allows extrapolation of shoot weight values based on whole plant weight experimental data in cases where no experimental data is available for control/solvent control shoot weight.

## **2.02.P-We048 A Comprehensive Laboratory Comparison of Two Methods for Generating Data for Green Algae Response to Time-Variable Exposures**

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The use of Toxicokinetic-Toxicodynamic (TKTD) modelling in European risk assessments for plant protection products is rapidly gaining momentum – especially following the 2018 publication of the EFSA opinion on TKTD modelling which states that several models are now ready for use in risk assessment. This report reviewed a number of TKTD models for primary producers, including the SAM-X model for green micro-algae. This model was considered not ready for use in risk assessment due to the lack of a robust and ring tested methodology for generating calibration and validation studies. In response, the present work funded by CropLife Europe (CLE) aims to establish robust and standardized guidelines for generating data for algae responses to time-variable exposures. The CLE project tackles two distinct methodologies – a flow-through system and a semistatic system. Each of these methods are evaluated by conducting a laboratory comparison on comprehensive parameters across six and eight independent laboratories respectively. We here provide a detailed overview of the project results to date and discuss the learnings for each of the two methodologies. Analysing the data across the laboratories allows us to refine the protocols, avoiding stumbling blocks experienced by the laboratories. We here present and discuss complications and difficulties experienced by the laboratories, and we provide guidelines for systematic and robust generation of this data in future based on the lessons learned from the laboratory comparison. The two methodologies are fundamentally very different, and we do not offer a direct comparison. Rather, we specify how each method may best be used in a risk assessment context following the guidelines laid down by EFSA. The data generation has been partly funded by CropLife Europe. The participating laboratories were provided some limited support in the form of materials, but the full cost of the labour was carried by each individual laboratory.

## **2.02.P-We049 Antifouling Coatings: Assessment of the Effects on Phytoplanktonic Nontarget Species**

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Biofouling is the settlement and growth of microorganisms, algae, plants, and/or animals on wetted artificial surfaces. To prevent the settlement and growth of these communities, antifouling coatings are commonly applied. Despite being biocide-based coatings (BC) the most widespread coatings, there is some uncertainty regarding their actual effectiveness or their potential indirect environmental impact. Thus, new commercially available alternatives, such as foul-release (FR) coatings, are being developed. This study aimed to test the effects of the two antifouling typologies in nontarget species, exposing selected phytoplankton species to different concentrations of paint lixiviates in lab conditions. PVC panels with a standardized surface were coated with BC and FR coatings, submerged in 1 L filtered and sterilized sea water and placed on a shaker for 24 h. The solutions were used to expose cultures of three different phytoplankton species (*Isochrysis galbana*, *Tetraselmis chuii*, and *Cylindrotheca* sp.) to different lixivate concentrations, each of which had 3 replicates, following OECD guidelines with small modifications. Exposure was carried out for 72 h and growth was measured every 24 h. After 72 h, samples from each culture were taken to measure 1) photosynthesis efficiency, 2) pigment content, and 3) oxidative stress through biochemical biomarkers (CAT, GST, and lipid peroxidation). Extra samples were taken for potential further analysis on transcription levels of HSP and metallothioneins. Exposure to BC lixiviates significantly affects the growth of the three species, being *T. chuii* the only species that recovered and grew after 48 h of exposure to the highest (25%) BC lixivate concentration. As regards the photosynthesis efficiency, the fluorescence yield (Fv/Fm) particularly dropped for BC-exposed *Cylindrotheca* cultures, but not for the other two tested species. In all studied species, FR-exposed cultures performed without differences to the controls. Further biochemical analysis will provide insights of physiological mechanisms that could also contribute to a greater resistance to these components.

## **2.02.P-We050 Method and Performance Evaluation in a Ring Test: Algal Growth Inhibition Test with Time-Variable Exposures**

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The Algal Growth Inhibition Test according to OECD Guideline 201 is an essential part of the ecotoxicological risk assessment of plant protection products. However, the test system is limited in different exposure scenarios and does not reliably reflect real environmental conditions due to the static single exposure. To better include important factors such as multiexposures, dissipation, or recovery times, we performed algae growth inhibition tests in flow-through bioreactors using time-varying exposure regimes and combined the experiments with a SAM-X *in silico* toxicokinetic/toxicodynamic (TK/TD) model for green microalgae. This innovative test setup combined with a modelling approach provides a deeper insight into population effects and thus might refine future Tier 2C risk assessments for primary producers. We will present the feasibility and some exposure

scenarios of the flow-through system for model validation and calibration based on our participation in the CropLife Europe ring test using *Raphidocelis subcapitata* and an herbicide.

## 2.02.P-We051 The Effect of Sodium Bicarbonate on Key Algal Species in the Static, Closed System Test Design

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In algal toxicity studies (OECD 201), one avenue for testing volatile test substances is utilizing the static, closed system, in which headspace is kept to a minimum or eliminated during preparation and exposure of the test organism. By doing so, this retains the test substance in the aqueous phase and prevents the test substance from entering the vapor phase and escaping the test system. Due to the removal of gas exchange, growth of algal test organisms in this test system can be limited by carbon dioxide depletion. The test guidance (OECD 23) suggests overcoming this issue by decreasing inoculation volume and supplementing the test solution with sodium bicarbonate. The test guidance is not specific as to what inoculation volume should be used or how much sodium bicarbonate should be added to the test medium. Most algal mediums outlined in the test guidance inherently have 15 mg/L sodium bicarbonate, with the exception of saltwater algal medium, which does not contain sodium bicarbonate. The purpose of this work is to elucidate the effect of varying concentrations of sodium bicarbonate on algal growth in a closed, static system. To explore the varying response, key algal genera such as *Raphidocelis*, *Navicula*, *Anabaena*, and *Skeletonema* will be investigated. These genera are commonly used in species sensitivity distribution tests and are recommended species in the OECD 201 guideline. This study design utilizes replicates that will be sacrificed daily to take samples for cell counts without introducing headspace into the test vessel. Mean cell density, growth rate, and yield will be measured to quantify the effect on the changing concentrations of sodium bicarbonate on cell growth. Growth will be evaluated based on significant difference ( $p < 0.05$ ) of the test groups. Preliminary results from studies conducted by our lab have indicated that algal species respond differently to trends in sodium bicarbonate concentrations within the test medium. Algal toxicity studies with volatile test substance are technically very difficult and may produce inconsistent results. Results presented will assess whether modifications to the carbonate system will improve reliability and reproducibility of algal toxicity tests conducted within a closed system.

## 2.02.P-We052 Evaluation of the Reproducibility, Reliability, and Regulatory Relevance of Plant Visual Injury Assessments

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In nontarget terrestrial plant (NTTP) studies (OECD 208 2006a and OECD 227 2006b) quantal data (e.g., survival) and continuous data (e.g., biomass) are assessed to estimate an  $ER_{50}$  with linear and nonlinear regression models, respectively. In the EU the  $ER_{50}$  is used to assess the risk of plant protection products (PPP) on NTTP and define potential restrictions of the products uses. The EFSA Technical Report (2019) proposes to consider also an  $ER_{50}$  based on visually assessed plant injuries, such as chlorosis, necrosis, wilting, etc., for the risk assessment if this is lower compared to others. The visual injury assessments are qualitative scores which are assigned to treated plants by comparison with untreated control plants. The NTTP guidelines do not provide adequate guidance as to which scoring system is to be used and how this should be applied to assess plant visual injuries in NTTP studies, e.g., it is not specified which and how many symptoms should be considered in one score and whether scores should be assigned per plant or per replicate. Also, there is no advice provided on how a statistically sound  $ER_{50}$  can be derived from this data. The assigned scores are ordinal data which do not allow for a statistical estimation of an  $ER_{50}$  as it is done for continuous or quantal data. Nevertheless, some countries in the EU request an  $ER_{50}$  for visual injury data to assess the risk of PPP. The SETAC Visual Injury Working Group (WG) was established to overcome these discrepancies between the limits of the methodology for the visual injury assessments and what authorities expect it to deliver. To evaluate and improve the reproducibility, reliability, and regulatory relevance of plant visual injury endpoints the WG will provide a detailed problem formulation. This will highlight the challenges of the methodology of visual assessments in the lab, the statistical evaluation of the data, and discuss the meaning of the data in the regulatory context. Based on these findings a definition of relevant criteria for a suitable scoring system will be possible to provide data that can be used for an  $ER_{50}$  estimation. This will serve as a base for a harmonization and standardization of the visual injury assessments for regulatory purposes. Further research on the implementation of reliable scoring systems and potential statistical evaluation with, e.g., Rao-Scott Cochran-Armitage by Slices needs to be evaluated in future.

## 2.02.P-We053 Biocide, Antioxidant, and Anti-Inflammatory Activities of Essential Oils Distilled from Aromatic Plants Grown on Trace Elements Polluted Soils

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Essential oils (EO) from coriander (*Coriandrum sativum*), sage (*Salvia sclarea*), and angelica (*Angelica archangelica*) were investigated for their biocide properties (antifungal and herbicidal) and their antioxidant and anti-inflammatory activities.

The novelty of this work lies in the integration of the principles of the circular economy (self-financing of the system) for the rehabilitation of polluted soils. Indeed, the essential oils producing plants under study were grown at Metaleurop Nord, a site highly contaminated by heavy metals, in the presence or absence of mycorrhizal inoculum. After the recovery of the different plants, essential oils were extracted and tested for their biological properties.

Antifungal properties were assessed through *in vitro* direct contact and volatility assays, demonstrating that all EOs present a fungistatic activity. Similarly, direct contact herbicidal assays have highlighted an herbicidal activity of all EOs, which may be used as biopesticides.

Antioxidant activities of essential oils were assessed by the scavenging of the diphenylpicrylhydrazyl (DPPH) radical method and anti-inflammatory potency was investigated through their ability to reduce inflammatory response induced by atmospheric fine particles (PM<sub>2.5</sub>) in human bronchial epithelial cell lines (BEAS-2B). Antioxidant effect was found for all EO but angelica and coriander leaves showed stronger antioxidant activity than coriander seeds and the sage. For the anti-inflammatory investigation, the same conclusion on the EOs from angelica and coriander leaves was highlighted as these two oils were able to counteract the induction of inflammation.

## **2.02.P-We055 Impacts of Petroleum-Based and Plant-Based Plastic Particles on Dry Acidic Grassland Plant Species**

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Microplastics are ubiquitous in all environments and plastic pollution has now become a new major environmental issue. Recent studies have pointed to agricultural fields as major terrestrial sink of microplastics. Grasslands are reservoirs of biodiversity, especially plants species. Great part of European grasslands originate from previously arable land, which raises concerns over the future of restoration and the improvement of grasslands, biodiversity and ecosystem functioning. The effects of land-use change, invasive species and climate change on grasslands is relatively well understood, however the study of the impacts of micro- and nanoplastic accumulation is still in its infancy. This novel, topical, and multidisciplinary study aims at understanding the effects of a range of petroleum-based and plant-based micro- and nanoplastics on plant physiology and growth from a grassland perspective. For this, plastic particles will be spiked into soil where standard grassland plant species will be sown and grown for 8 weeks. Nanoplastics will be administered through their watering cycles. The results from this novel study will be presented especially focusing on the impacts on key parameters in plant development, such as shoot and root growth. Also, microplastic adsorption to roots and nanoplastic intake in plant tissue will be examined. Overall, this study will inform about the different ecotoxicological effects of a range of plastic particles on terrestrial plants.

## **2.02.P-We056 Temperature Effects on the Toxicity of Triclosan to Oilseed Rape (*Brassica napus* L.)**

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There is growing concern about the environmental effects of antimicrobial chemicals because of their increasing levels in the environment and lack of information about their ecotoxicity. Triclosan (TCS) is one of the most widely used antimicrobial agents in both pharmaceuticals and personal care products, and it is a frequently detected emerging organic contaminant in the environment. Reported studies of TCS toxicity to terrestrial plants mostly rely on whole-organism morphological or physiological endpoints. There is a need for more profound studies about TCS-induced effect at biochemical plant level to discover underlying toxicity mechanisms. Moreover, climate change is an increasingly urgent problem with potentially far-reaching consequences for life on earth. Little is known about the effect of climate parameters on the ecotoxicity of the antimicrobials, including TCS. The main objective of this study was to evaluate temperature effect on triclosan toxicity to oilseed rape (*Brassica napus* L.). *Brassica napus* was grown in TCS contaminated soil (10-400 mg kg<sup>-1</sup>) under different temperatures (21°C and 25 °C). *B. napus* morphological (dry weight, length of the roots, and shoots), biochemical indicators (the activity of enzymes), and the damage of oxidative stress (lipid peroxidation) were detected.

## **2.02.P-We057 Development of a Standard Protocol for the Assessment of Reproductive Endpoints in Nontarget Terrestrial Plants under Greenhouse Conditions**

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Ecotoxicological testing to assess the effects of plant protection products on nontarget terrestrial plants (NTTPs) under greenhouse conditions is conducted according to standard guidelines (e.g., OECD 208 and OECD 227). EFSA's Scientific Opinion of 2014 on the risk assessment of plant protection products (PPP) for nontarget terrestrial plants raised awareness of a potential need to assess effects on plant reproduction, e.g., seed production.

Literature reviews and experimental studies evaluated the feasibility and relevance of plant reproductive endpoints (e.g., Christl et al. 2020, Duffner et al. 2020). In summary, they concluded that (a) assessment of reproductive endpoints in selected plant species is in principle feasible, (b) there is no clear trend that reproductive endpoints are more sensitive than vegetative ones, and (c) specific cases may exist where reproductive endpoints appear to be more sensitive depending on e.g., mode of action of a plant protection product and/or test species.



For cases where effects on reproductive endpoints need to be assessed for regulatory purposes, a standard test protocol is required. Thus, a working group within the SETAC Plant Interest Group was launched in November 2021 to collect and analyze relevant and available data and experiences in order to provide guidance for a plant reproduction protocol. The working group meets regularly in three subgroups covering (a) Plant species selection, (b) Challenges & Restrictions, and (c) Protocol elements to develop the protocol.

The objective of this poster is to create awareness of the topic, to provide an overview and status of this working group, and, in particular to present some of the challenges and foster a discussion on possible solutions in order to obtain a broadly acceptable standard test protocol.

## **2.02.P-We058 Changes in Antibiotics Levels After Treating Poultry Litter and Their Effects on the Early Stages of Chicory Seedling Development**

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Poultry litter (PL) is used as fertilizer because it contains essential nutrients for crops. However, it has proven to be an important reservoir of pollutants, especially antibiotics (ATBs). ATBs can be accumulated in soils, being taken up by edible plants, entering the food chain with unknown environmental and human health risks. Thus, this work seeks: (1) to characterize the ATBs present in raw and treated PL to verify their drop upon self-heating processes; (2) to study the effect of treatment + ATBs on the germination of chicory seeds, verifying the phytotoxicity of treated PL containing ATBs on the early stages of seedling development. Thus, we used five methods of treatment: T1-Pile without cover and without turning; T2-Pile with cover and without turning; T3-Pile with cover and with turning; T4-Mini pile with cover and with turning; and T5-Pile without cover and with turning. ATBs in PL were extracted by salt-assisted solvent extraction and quantified by UPLC-MS/MS. Phytotoxicity assays were carried out by diluting samples (raw PL and T1-T5) with ultrapure water at three proportions (10, 50 and 100 g/L). Seeds were added to Petri dishes containing filtration paper and control and PL solutions (raw and T1-T5), and further incubated in the dark for 6 days (in triplicate). After 6 days of incubation, seedlings were harvested and dissected. Germination rate and lengths were recorded. Cotyledons, shoots, and roots from each Petri dish were pooled and dried to measure dry biomass, moisture, and specific lengths. Nine antibiotics were found in PL samples, being higher in raw PL but decreasing upon treatment in the following order: T4>T3>T2>T1>T5. Phytotoxic assays showed that all the samples at 50 and 100 g/L decreased the biomass of root, and both length and specific length of root and shoot with respect to control. T3 and T4 seem to be the least effective treatments to eliminate ATBs, showing the highest phytotoxicity with respect to other treatments, being the root and shoot parameters the most affected by the presence of ATBs in PL (average correlation -0.67,  $p < 0.05$ ). Thus, this study confirms that ATBs have negative effects on the early stages of chicory seedling development. Our current results also demonstrate that different PL treatments have diverse efficacy to eliminate them from polluted PL. Therefore, finding a good treatment to remove ATBs and other pollutants from PL looks needed to avoid negative effects on plants growing in amended soils.

## **2.02.P-We059 Intralaboratory Variability of Visual Phytotoxicity Assessments in Nontarget Terrestrial Plant Studies**

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The EFSA recurring issues in ecotoxicology guidance from 2019 indicates that “the EC<sub>x</sub> based on phytotoxicity should be reported” and “it should be considered for the Tier 1 risk assessment” in case it is the lowest endpoint. As visual estimates may have several sources of imprecision and bias, aspects of the reproducibility of the visual phytotoxicity assessments for possible derivation of ER50 values and their use in the European risk assessment of non-target terrestrial plants (NTTP) have been evaluated in this vegetative vigour study.

Five plant species (onion, oilseed rape, soybean, sugar beet, tomato), typically used in NTTP studies, were sprayed with two herbicides with different modes of action. An untreated control and five treatments (from 0.0625× to 1× of their field use rate) were assessed for each herbicide. Phytotoxicity was recorded 21 DAT and evaluated based on a total of 1227 photos of the plants. Twenty three experienced assessors from 10 labs assigned phytotoxicity scores to the plants based on the photos blinded to the treatment. For seven labs, 2-4 assessors participated and assigned scores independently from their colleagues. The data from these seven labs was used in this analysis. As staff working at the same company can be assumed to have received similar training and/or is operating to the same internal guidance, the intralaboratory variability of the scores assigned to the plants was evaluated.

The alignment in scores recorded by assessors from the same company was determined using the Intraclass Correlation Coefficient (ICC), preliminarily for three of the plant species considered in the study: tomato, sugar beet, and onion.

For the scoring of tomato and onion, the analysis showed a high intralaboratory variability between assessors, except for one laboratory where consistent scoring was provided by the observers. A much higher intralaboratory consistency was found in the visual phytotoxicity scores assigned to sugar beet plants.

Overall, inconsistent assessments among staff were observed at six of the seven laboratories that participated in the study, especially for some species commonly used in NTTP tests (e.g., onion and tomato). The use of ER<sub>50</sub> values based on visual phytotoxicity scoring in the European ecotoxicological risk assessment for NTTP needs to be carefully considered.

#### **2.02.P-We060 Determine Endpoints on Visual Effects for Terrestrial Plant (NTTP) Risk Assessment**

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Besides emergence, biomass, and shoot length, authorities ask to determine ER<sub>50</sub> values for visual effects to conduct terrestrial plant (NTTP) risk assessment for plant protection products (PPP). Guidelines for terrestrial plant testing (OECD 208 and 227) provide some general guidance to assess visual effects (also referred to as “visible detrimental effects” or “visual injury,” such as chlorosis, necrosis, wilting, leaf and stem deformation). However, the assessment of the data on visual effects presented in the studies for authorisation process is not always documented in a way to readily conclude on the ER<sub>50</sub> needed for NTTP risk assessment. Although presentation of the effects on visual injury as percent compared to the untreated control is recommended for the assessment of visual phytotoxicity in the above-mentioned guidelines, a number of studies provide an alternative scoring system. We present a scheme on how to derive effect endpoints (ER<sub>50</sub>) for different data set types and give advice for appropriate statistical methods. To shed light on the uncertainties of the different procedures, statistical analyses were conducted using the examples of ER<sub>x</sub> and Cochran-Armitage approach.

We want to share this proposal to facilitate the determination of the endpoint “visual injury” from the studies used to evaluate the risk of PPP to NTTPs and to thereby support the evaluation of such data for PPP risk assessment.

#### **2.02.P-We061 Terrestrial Plant Species Sensitivity Distribution Approaches for Veterinary Medicinal Products**

**Chris Sinclair**, Roy Macarthur and Nick Jarratt, Fera Science Ltd., York, United Kingdom

Veterinary medicines may enter the terrestrial environment through the application of livestock manure and slurries to land as a soil improver, or through direct defecation by treated animals. As part of the environmental risk assessment for these compounds the risk posed to terrestrial taxa including higher plants needs to be considered. Specific guidance on assessing the risks to terrestrial plants is available and follows a tiered process where initially acute data is used to generate the predicted no-effect concentration (PNEC). Usually the lowest EC<sub>50</sub> from an OECD208 seedling and emergence study is used which considers six plant species (from six families, including two monocotyledonous and four dicotyledonous species including a Brassica). Should the risk be unacceptable then chronic data (NOEC or EC<sub>10</sub>) usually from the same study can be used to refine the PNEC and thus refine the risk. Should the risk still be unacceptable then there are higher tier options available, one of which is the use of a species sensitivity distribution (SSD) to refine the PNEC.

The establishment of the SSD requires a minimum of eight species and using data for ‘the same, most sensitive endpoint’ an SSD can be established and the PNEC set as the lower confidence level concentration at which 95% of the species are theoretically protected (i.e., HC<sub>5</sub> LL). Two approaches are recommended within the EMA guidance for the establishment of the SSD (RIVM ETX and EPA CADDIS). We have explored the use of these approaches and we have identified that they do not generate the same HC<sub>5</sub> LL and hence PNEC and can vary significantly. In the presentation we will demonstrate the difference in PNEC values generated for a range of veterinary medicines using the two approaches based on real plant ecotoxicological data, we will describe the main statistical differences underlying the approaches and, through simulation modelling demonstrate how the approaches differ. Finally, we will make a recommendation on the use of an SSD modelling approach for higher tier plant environmental risk assessment for veterinary medicines.

#### **2.03.P Aquatic Model Ecosystems and Aquatic Ecosystem Models: How Can They Be Used to Support Ecological Risk Assessment of Chemicals?**

##### **2.03.P-We062 Mesocosm Studies: Representativeness and Reliability**

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In recent years the representativity and reliability of mesocosm data for regulatory risk assessment has been challenged, with some research suggesting that the organism diversity and abundance found in mesocosms do not represent the same levels typically found in natural waterbodies. In addition, some mesocosm studies have been criticised for their low statistical power to detect treatment related effects.

Despite these criticisms, mesocosms remain a valuable option in the risk assessment toolkit and recent improvements in study design and ecological representativity provide evidence that fresh concerns may be unwarranted.

In this poster we will provide evidence from recent mesocosm studies to demonstrate that when compared to natural edge-of-field waterbodies, mesocosms can provide a conservative, and protective, tool to evaluate the effects of pesticides to freshwater communities in different water body types (streams, ponds and ditches). Furthermore, we will show how mesocosm study designs can be informed by lower tier data to comprise sensitive and vulnerable organism groups, at abundances that allow robust statistical endpoints to be determined.

Through this analysis, we aim to demonstrate that data from mesocosm studies can be representative of the natural waterbodies they are attempting to emulate. These data will be supported by statistical (MDD) analysis to highlight the reliability and relevance of higher tier mesocosm studies in environmental risk assessment.

### **2.03.P-We063 Towards a Virtual Mesocosm for Pesticide Risk Assessment: A Comparison of Four Models Applied to Mesocosm Data**

Chiara Accolla<sup>1</sup>, Amelie Schmolke<sup>1</sup>, Nika Galic<sup>2</sup>, Steven M. Bartell<sup>3</sup>, Daniel Dawson<sup>4</sup>, Klaus Peter Ebke<sup>5</sup>, Jana Gerhard<sup>6</sup>, Ann-Kathrin Loerracher<sup>5</sup>, Isabel O'Connor<sup>7</sup>, Laura Mudge<sup>4</sup>, Robert Pastorok<sup>4</sup>, Damian V Preziosi<sup>4</sup>, Brandon Sackmann<sup>8</sup>, Juergen Schmidt<sup>5</sup>, Nele Schuwirth<sup>9</sup>, Tido Strauss<sup>6</sup> and Roman Ashauer<sup>2,10</sup>, (1)Waterborne Environmental, Inc., (2)Syngenta Crop Protection, Basel, Switzerland, (3)Stantec, Canada, (4)Integral Consulting, Inc., (5)MESOCOSM GmbH, Institut für Gewässerschutz, Germany, (6)gaiaac - Research Institute for Ecosystem Analysis and Assessment, Aachen, Germany, (7)EBP Schweiz AG, Zürich, Switzerland, (8)GSI Environmental, Inc., (9)Systems Analysis and Modelling, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, (10)Department of Environment and Geography, University of York, York, United Kingdom

Aquatic mesocosm studies are used in higher-tier aquatic risk assessments of pesticides. Aquatic systems models (ASMs) could be used to interpret and extend mesocosm experiments, e.g., by modeling ambient environmental and pesticide exposure conditions untested in the empirical systems. Mesocosm studies can provide data for aquatic systems models (ASMs), which are rarely available for natural systems. Thus, ASMs could become valuable tools to complement higher-tier aquatic risk assessments.

We present the results of a comparative modeling ("ring") study with four previously published ASMs (Streambugs, AQUATOX, CASM, and StoLaM+StreamCom). In the project, the four models were calibrated and validated to mesocosm control and toxic effect data conducted by Mesocosm GmbH at FNU Research Centre Neu-Ulrichstein, Germany. In the treatment studies, a fungicide was applied.

We reviewed the data for patterns and consistencies across studies and derived control and effect calibration and validation criteria for the ASMs applicable across studies. The ring study revealed uncertainty and data gaps related to the characterization of the mesocosm systems necessary for ASM parameterizations and evaluation of model performance. The calibrated ASMs met the predefined control calibration and validation criteria to varying extents. Each model performed differently in describing the abundances and dynamics of the organism groups and taxa in the food web. Organism groups best represented in simulations varied among the four models. In the effects simulations, the ASMs captured the most pronounced treatment-related effects across the study period. Some models successfully represented indirect effects resulting in increased taxon abundances in the mesocosm studies.

This study is a crucial first step toward the use of ASMs as 'virtual mesocosms' that can inform higher-tier aquatic risk assessments. The application of ASMs in the context of higher-tier aquatic risk assessments in the European Union can potentially extend the use of data from these studies by applying primarily different exposure profiles, but ultimately also environmental conditions and species compositions.

### **2.03.P-We064 Use of Hybrid Ecosystem/IBM Models to Mimic Outdoor Aquatic Mesocosms for Pesticide Risk Assessment**

Tido Strauss<sup>1</sup>, Natalie Carmen Dallmann<sup>1</sup>, Josef Koch<sup>1</sup>, Juergen Schmidt<sup>2</sup>, Chiara Accolla<sup>3</sup>, Amelie Schmolke<sup>3</sup>, Nika Galic<sup>4</sup> and Roman Ashauer<sup>4</sup>, (1)gaiaac - Research Institute for Ecosystem Analysis and Assessment, Aachen, Germany, (2)MESOCOSM GmbH, Institut für Gewässerschutz, Germany, (3)Waterborne Environmental, Inc., (4)Syngenta Crop Protection, Basel, Switzerland

Aquatic mesocosms are artificial ecosystems regularly used as proven test systems for higher-tier risk assessments of pesticides. At the same time, mechanistic effect models are also considered useful tools for ecological risk assessment of chemical stressors. In recent years, individual-based population models (IBMs) have been increasingly used in ecological risk assessment, often coupled with TKTD effect models. Integrated within ecosystem models, they can be powerful tools for predicting species population dynamics for different environmental scenarios.

This study is part of a larger ring study comparing four aquatic system models (ASMs) with different model structures to evaluate whether such modelling approaches can be used to simulate aquatic mesocosms. The ring study is a step toward the use of ASMs in the regulatory context of pesticide risk assessment in the future. Here we present one of the four ASMs, which is the hybrid ecosystem model framework StoLaM+. StoLaM+ links the lake model StoLaM, a hydrodynamic-ecological compartment model including several zooplankton and phytoplankton species, and IBMs for pelagic taxa such as daphnids, copepods and the dipteran *Chaoborus crystallinus*. Model dynamics are driven by year-specific meteorological weather data. The IBMs incorporate TKTD effect models for dynamic simulation of pesticide effects. Data from several outdoor aquatic mesocosm studies with and without pesticide treatment were available for several calibration and validation steps of the models.

This presentation focuses on the pelagic food web, where the most pronounced direct effects of the applied fungicide were found on planktonic crustaceans. The degree of adaptation of the model parameters to the respective case study is essential for the simulation quality as well as the transferability of the model to deviating ecological scenarios. In this application example, most of the parameters of the StoLaM+ model were kept unchanged and only study-specific parameters and pesticide- and organism-specific parameters of the effect models were calibrated. We derived validity criteria for the simulation quality from the minimum detectable differences (MDD values) in the mesocosm study and the deviation between treatments and controls over the

experimental time. Simulated direct and indirect effects of the fungicide on the pelagic organisms are presented and discussed in comparison with the effect data of the experimental mesocosm studies.

### **2.03.P-We065 Process-Based modelling of Shallow Aquatic Microcosms Reveals Adaptation of Phototrophic Communities to Agricultural Run-off and Warming**

**Bastian H. Polst<sup>1</sup>**, Grégorio López Moreira Mazacotte<sup>2</sup>, Elisabeth Maria Gross<sup>3</sup>, Mechthild Schmitt-Jansen<sup>1</sup>, Franz Hölker<sup>2</sup> and Sabine Hilt<sup>2</sup>, (1)Department of Bioanalytical Ecotoxicology, Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany, (2)Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany, (3)LieC Cnrs Umr 7360, University of Lorraine, France

Surface run-off transports nutrients and pesticides from agricultural sites into nearby shallow aquatic ecosystems that are dominated by different primary producer groups: submerged macrophytes, periphyton or phytoplankton. Agricultural run-off (ARO) can induce shifts from a macrophyte-dominated to a phytoplankton-dominated state, which may alter or impair their ecological functioning and services. Global warming may further increase the risk of these ARO-induced shifts. A microcosm experiment mimicking ARO effects on shallow aquatic systems with different primary producer groups was combined with process-based modelling to test three hypotheses: 1) Differences in the vulnerability of different primary producers to ARO increase the risk of shifts to a phytoplankton-dominated state; 2) warming further increases this risk; 3) process-based modelling supports mechanistic understanding of ARO effects on primary producers through comparison of different scenarios.

A simplified shallow aquatic system containing different primary producer groups (phytoplankton, periphyton, submerged macrophytes) was exposed to a gradient of ARO (five different concentrations of a mixture of nitrate, an herbicide, an insecticide, a fungicide, copper) at two different temperatures, 22°C and 26°C, for three weeks. A tailor-made process-based model was used to test eight different scenarios, targeting different temperature-dependent growth rates, herbicide sensitivities and community adaptation to pesticides.

In the experiment ARO had negative effects on macrophytes while phytoplankton was positively affected. Warming further enhanced phytoplankton growth. Comparing model scenarios with the observed results revealed that the best quantitative and qualitative fit was achieved when community adaptation of phytoplankton was considered with lower herbicide sensitivity at higher temperature.

Our study highlights the benefits of combining process-based modelling with experiments to disentangle and mechanistically understand effects of multiple stressors in shallow aquatic systems. Warming and ARO were shown to potentially lead to a shift from macrophyte dominance to phytoplankton dominance, and fast phytoplankton community adaptation to pesticides contributed to this effect.

### **2.03.P-We066 The Interplay Between Pollutants and the Adaptive Behavior in Ecological Communities**

**Constanza Vega Olivares<sup>1</sup>** and Andreu Rico<sup>2</sup>, (1)IMDEA Agua, Spain, (2)IMDEA Water Institute, Spain

The relationship between the adaptive responses of predators and the stability of communities has been previously analyzed only considering pristine environments, even though many ecological systems are exposed to pollutants, among other disturbances. Many pollutants tend to accumulate in the tissues of organisms, resulting in species present in trophic webs not only transmitting their effects among themselves, but also transmitting the pollutants through their trophic interactions. In this context, adaptive behaviour, considered in this study as changes in prey preference, can inhibit pollutant transport through trophic networks without completely limiting biomass flow. In this study, the effectiveness of adaptive responses of predators in promoting the persistence and diversity of species in trophic networks subjected to stress from a bioaccumulative pollutant was evaluated. A community model based on the bioenergetic model was used, where the effects of the network topology (species richness and connectivity), the pollutant concentration and the adaptive behaviour over the species persistence and Shannon-Wiener diversity index were distinguished. In this work it was shown that adaptive behavior promotes species diversity in communities subject to stress from a bioaccumulative pollutant but does not promote species persistence. Therefore, the adaptive responses of predators have a beneficial role on the diversity of species in trophic webs affected by bioaccumulative pollutants.

### **2.03.P-We067 Towards a Virtual Mesocosm: Using the CASM-cosm to Assess Pesticide Risks**

**Steven M. Bartell<sup>1</sup>**, Chiara Accolla<sup>2</sup>, **Amelie Schmolke<sup>2</sup>**, Cindy Woodard<sup>1</sup>, Peter Ebke<sup>3</sup>, Nika Galic<sup>4</sup> and Roman Ashauer<sup>4</sup>, (1)Cardno, Inc., (2)Waterborne Environmental, Inc., (3)MESOCOSM GmbH, Institut für Gewässerschutz, Germany, (4)Syngenta Crop Protection, Basel, Switzerland

The comprehensive aquatic systems model (CASM) is a bioenergetics-based food web/ecosystem model used previously to assess pesticide risks in experimental ponds, lower-order streams, off-channel habitats, and wetlands. The CASM was adapted to simulate the FNU Research Centre outdoor pond mesocosms located near Neu-Ulm, Germany. The resulting CASM-cosm included multiple populations of periphyton, phytoplankton, zooplankton, and benthic macroinvertebrates designated as important based on available control mesocosm data. The growth of the modeled populations was determined by parameters derived from the technical literature and previous CASM applications and physical-chemical data specific to each mesocosm study. The CASM-cosm was calibrated to four FNU mesocosm study control data sets. Calibration was performed by manually adjusting key growth parameter values (e.g., rates of photosynthesis, consumption, respiration) and comparing modeled to observed abundance and associated ASM ring study calibration criteria for the modeled populations. The calibrated model was within the range of the calibration criteria for 88 percent of the possible comparisons for the modeled macroinvertebrates and 71 percent for modeled

zooplankton. The calibration matched 10 percent of the comparisons for modeled phytoplankton and 13 percent for periphyton. The control calibration was subsequently calibrated to population abundance data for mesocosms treated with the pesticide azoxystrobin (AZT). The calibration to measured effects of AZT was performed by deriving toxicity benchmark values (e.g., EC50 values) for each modeled population. Values of the model inputs used in the calibration to the control data were not changed during calibration to the AZT mesocosm data. Repeated simulations with systematically adjusted EC50 values were performed to match the magnitudes and temporal patterns of population abundances reported for the treated mesocosms. Daily values of control and treatment population biomass were plotted for the 365-day simulations. Percentage impacts (+/-) on abundance were plotted in relation to minimum detectable differences defined by the variability of population responses among replicate (N = 3) treatment mesocosms. Average differences between calibrated model and measured percentage impacts ranged from 10 for nauplii to 83 for small Cladocera. The CASM-cosm provides a virtual representation of the FNU mesocosms for higher-tier pesticide risk assessments.

**2.03.P-We069 Seasonal Variability Physicochemical Parameters in the Mesocosm System Located in Southern Poland**  
**Katarzyna Winiarska<sup>1</sup>, Inga Mrzyk<sup>2</sup>, Agnieszka Godziek-Botor<sup>2</sup>, Katarzyna Brzozowska-Wojczech<sup>2</sup>, Anna Wróbel<sup>2</sup>, Agnieszka Żmijowska<sup>2</sup> and Marcin Wójcik<sup>2</sup>, (1)Institute of Industrial Organic Chemistry, Poland, (2)Łukasiewicz Research Network-Institute of Industrial Organic Chemistry Branch Pszczyna, Poland**

In 2020, nearly 25 thousand tons of plant protection products (in terms of active substance) were sold in Poland. Most of them, i.e., 56%, are herbicides, weed destructors and moss killers, 32% are fungicides, 5% are insecticides, 4% are plant growth regulators, and 3% are others. Plant protection products have been known for decades and there is no doubt that they play an essential role in maintaining high level food production, but on the other hand, they affect the aquatic ecosystem and many biological processes involved.

In view of this fact, performing an outdoor mesocosm study for environmental risk assessment appears to be of ever-greater importance. Studies in the mesocosm system are designed to simulate the natural aquatic ecosystem, which can be created in the form of independent tanks or separate parts in existing ponds. During the study, physicochemical analyses of water are performed in regular periods of time. An extremely important element is the proper preparation of the mesocosm system before starting the experiment. Based on this, the above described study was performed, the aim of which was to conduct the research determining the physicochemical structure of the ecosystem over two growing seasons. In 2020 and 2021, the individual tanks with aquatic ecosystem established in 2019 were subjected to a number of physicochemical tests. Physical and chemical parameters of water such as pH, temperature, hardness, turbidity, conductivity, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>-</sup>, TOC were determined. The obtained results are a set of historical data for the mesocosm studies.

**2.03.P-We070 Aquatic Risk Assessment for Nonstandard Chemicals with Physical Mode of Action (Forming a Film at the Water Surface) and Mesocosm Data - 2D and 3D**

**Heino Christl and Nicole Santos, Tier3 Solutions GmbH, Germany**

Standard aquatic RA assumes solubility of substances in water, and compares predicted concentration in the field with effect concentrations. There are however substances not miscible with water, and despite of some emulsifier in the formulation the spray mist of a drift event settles on the water surface, where it may form a film if the amount per area is high enough. This film can cut off the atmospheric air supply for aquatic insects, if it is thick enough and continuous. In the case presented here, these aquatic insects were assessed in mesocosm studies with realistic spray applications, mimicking spray drift. Also predicted environmental exposure focusses on spray drift – as agreed with regulatory authorities. Hence both exposure levels and effect levels can be expressed as rates, and be compared directly - two-dimensional approach (2D), applying the usual assessment factor. The standard procedures in aquatic risk assessment are three-dimensional (3D), comparing exposure and effect concentrations, which is appropriate for most organisms. For the surface dwellers discussed here, concentrations are purely theoretical (virtual – nominal concentrations), as in reality the substances act as a surface film. Due to different depths considered in e-fate and ecotoxicology (30 cm depth assumed in e-fate, 75 and 100 cm tested in the ecotoxicological experiments), the virtual concentrations that would be used in a standard risk assessment are not representative of these not-miscible substances and surface-dwelling organisms. Here a direct RA based on rates (mg/m<sup>2</sup>) would address the issue. If based on virtual concentrations (mg/L), a correction factor for water depths would have to be introduced. As an example, the case of Paraffin oils (CAS n°8042-47-5) will be presented in detail. This presentation may be relevant also for other substances under assessment, with very high Log K<sub>oc</sub>, and the specific organisms affected by films forming on the water surface.

**2.03.P-We071 Are Mesocosms Really Not suitable for the Risk Assessment of Plant Protection Products?**

**Udo Hommen<sup>1</sup>, Eric Bruns<sup>2</sup>, Klaus Peter Ebke<sup>3</sup>, Ivo Roessink<sup>4</sup>, Tido Strauss<sup>5</sup> and Nadine S. Taylor<sup>6</sup>, (1)Fraunhofer IME - Institute for Molecular Biology and Applied Ecology, Schmallingenberg, Germany, (2)Environmental Safety, Bayer AG - Crop Science Division, Germany, (3)MESOCOSM GmbH, Institut für Gewässerschutz, Germany, (4)Wageningen Environmental Research (WUR), Netherlands, (5)Research Institute gaiac, Germany, (6)Cambridge Environmental Assessments (CEA), United Kingdom**

A recently published article on the representativity of macroinvertebrate communities in micro- or mesocosm studies used as a higher tier tool in the environmental risk assessment of plant protection products (PPPs) in the EU concluded that micro-/mesocosm studies do not represent natural macroinvertebrate communities. We believe that this conclusion is unfounded for multiple reasons.

Fundamentally, the article based its conclusion on the analysis of studies in lentic micro- and mesocosms submitted to the UBA in a comparison to data from streams used as reference sites in a recent monitoring project in Germany for testing effects on typical stream taxa. Additionally, streams are not the only type of water bodies in agricultural landscapes and stream taxa are not, per se, more sensitive or vulnerable to pesticides than species living in ponds or ditches. Having initially identified 47 studies, the data used in the final analyses by Reiber et al. was constrained to eventually comprise of only 7 micro- or mesocosm studies, where mean numbers of taxa were considered by the Reiber et al. as sufficiently abundant for statistical evaluation. These were then compared against mean number of taxa present in at least five, of 22, streams. This appears to be a biased comparison.

Here we intend to revisit the data provided by the 7 selected studies from this publication, with the objective of determining how many, and which, taxa were considered as potentially sensitive or vulnerable and allowed a meaningful statistical analysis of effects with no other constraints. In our view, carefully designed and well conducted micro- and mesocosm studies do provide reliable and useful data for the prospective environmental risk assessment of PPPs, and other chemicals, since they are the only aquatic experimental option to cover long-term as well as indirect effects under semi-natural conditions. Whilst artificial streams offer an alternative to lentic systems if there is a special concern on typical stream taxa, it should be considered that lentic test systems provide additional safety for extrapolation to streams since the exposure events to tested in lentic systems are usually prolonged compared to the ones expected for pesticides in streams and the sensitivity of typical stream taxa can be checked in laboratory tests if needed.

### **2.03.PC Aquatic Model Ecosystems and Aquatic Ecosystem Models: How Can They Be Used to Support Ecological Risk Assessment of Chemicals?**

#### **2.03.P-We068 Effects of Herbicide Application on Early LIFE Stages of Fish: Connecting Laboratory and Field Work** **Gavin Dehnert<sup>1</sup> and William Karasov<sup>2</sup>, (1)VCGR, University of Wisconsin, Madison, (2)Forest and Wildlife Ecology, University of Wisconsin, Madison**

Invasive, non-native aquatic species like Eurasian watermilfoil (*Myriophyllum spicatum*) are rapidly spreading across the United States. Both established and new aquatic herbicides, such as 2,4-Dichlorophenoxyacetic acid (2,4-D) and florypyrauxifen-benzyl (FPB), are popular choices to combat and control invasive species due to their efficacy and ease. Aquatic herbicides are typically applied in the spring for maximum efficacy on their target species, however this timing corresponds with many early life stages of non-target organisms. Many aquatic herbicides active ingredients and degradants are stable in the water column leading to prolonged exposure throughout development. Recent laboratory research has shown that ecologically relevant concentrations of 2,4-D can negatively impact multiple species of freshwater fish species at different points during ontogeny; however, it is poorly understood whether these laboratory results accurately predict impacts in the natural lake environment undergoing standard practices of aquatic herbicide application. Therefore, first we investigated the effects of 2,4-D whole-lake treatments on the development and survival of fathead minnows in lakes undergoing treatment using mobile, in situ, and laboratory exposure systems. Fathead minnow larvae were exposed to either control lakes or lakes undergoing 2,4-D whole-lake treatment and effects on morphology, survival, and growth were evaluated. We found that exposure of fathead minnow larvae to EPA-permitted 2,4-D whole-lake treatments reduced survival as compared to controls matching previous laboratory results. Second, we conducted a pilot study to investigate the effects of a new aquatic herbicide, FPB, whole-lake treatment on the development and survival of fathead minnows in lakes undergoing treatment using in situ and laboratory exposure systems. We found that exposure of fathead minnow larvae to EPA-permitted FPB whole-lake treatments reduced survival as compared to controls in some lakes and had no effect in other lakes. Further laboratory research is needed to elucidate the field study results. Together, our results shed light on the broader environmental costs of aquatic herbicide application on non-target organisms, which can help inform data driven management decisions and improve risk assessment for non-target organisms.

### **2.04 Are the Sub-Individual Responses Translated into Effects to the Higher Level of Biological Organization?**

#### **2.04.T-01 Pollution-Induced Community Tolerance in Freshwater Biofilms – From Molecular Mechanisms to Loss of Community Functions**

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Aquatic biofilms include a variety of phyla, such as unicellular microalgae, bacteria, archaea, fungi, and protozoa, whose diversity and ubiquitous occurrence turns biofilms into a hotspot of ecological functions. Exposure to herbicides poses a threat to aquatic biofilms by affecting their community structure, physiology and function. These changes render biofilms to become more tolerant during exposure, but on the downside community tolerance has ecologic costs. We quantified microbial tolerance development against the herbicide diuron using the approach of pollution-induced community tolerance (PICT), analysed the molecular mechanisms of tolerance at the transcriptional and metabolic levels, and assessed its impact on ecological function.

We cultivated two different freshwater biofilm communities for a period of five weeks, with one set of the communities being contaminated with 4 µg L<sup>-1</sup> diuron. Subsequently, the communities were characterized for structural and functional differences, especially focusing on their crucial role of primary production. Further, the molecular fingerprints of the communities were assessed using meta-transcriptomics (RNA-Seq) and GC-based community metabolomics approaches and analyzed with respect to changes in their molecular functions.

Chronic exposure to diuron impaired freshwater biofilms in their biomass accrual, with particular reduction of autotrophic biomass. This loss was associated with a change in the molecular fingerprint of the communities and substantial structural and physiological changes. An increase in diuron tolerance has been detected in the contaminated communities and molecular mechanisms facilitating tolerance have been found. It was shown that genes of the photosystem, reductive-pentose phosphate cycle and arginine metabolism were differentially expressed among the communities and that an increased amount of potential antioxidants was found in the contaminated communities. This led to the hypothesis that contaminated communities may have adapted to oxidative stress, making them less sensitive to diuron. Moreover, the photosynthetic light harvesting complex was altered and the photoprotective xanthophyll cycle was increased in the contaminated communities. Despite these adaptation strategies, the loss of autotrophic biomass has been shown to impair primary production. This impairment persisted even under repeated short-term exposure, so that the tolerance mechanisms cannot safeguard primary production.

#### 2.04.T-02 Reduced Interspecific Competition Compensates the Costs of Pesticide Adaptation in a Dominant Aquatic Species

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Pesticide exposure alters invertebrate species composition in streams even below regulatory concentrations. Tolerant species are characterized by ecological traits enabling fast recovery of impaired populations. Additionally, pesticide tolerance enables their survival in contaminated streams. Tolerance can be increased through genetic adaptation; however, it involves fitness trade-offs. Here we show that pesticide exposure increased the pesticide tolerance, reduced the genetic diversity, resulted in an adopted genetic composition and reduced individual fitness. For 38 small streams we reveal that agricultural pesticide exposure resulted in loss of vulnerable species. For the less-vulnerable and dominant crustacean *Gammarus pulex*, an AFLP-assay comprised of 451 loci in 1035 individuals revealed an increased frequency of “high contamination alleles” and a decrease of “low contamination alleles” with increasing contamination. Furthermore, short geographical distance to nearby refuge sections reduced the tolerance development, but increased the genetic diversity and frequency of “low contamination alleles” presumably through individual migration. Finally, the individual per capita growth decreased with increasing trade-offs of acclimation and/or genetic adaptation. Nevertheless, *G. pulex* contributed an average of 44% of macroinvertebrate abundance and benefited from reduced interspecific competition with vulnerable species in contaminated streams. We conclude that pesticide contamination at already very low toxic pressure select for tolerant genotypes with less performant individuals and hypothesize that dominant species with high adaptive potential may nevertheless persist due to reduced interspecific competition with vulnerable species.

#### 2.04.T-03 Linking Individual Responses to Collective Outcomes: Does Exposure to Fluoxetine Disrupt the Collective Behaviour of Fish?

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Collective behaviours in wildlife, such as schooling in fish, are key traits shaping ecological communities and demographic processes. The performance and outcomes of such collective actions rely heavily on the interactions among group members. Yet, whilst exposure to pharmaceutical pollutants has been shown to elicit a range of behavioural effects on individual organisms, research on how these individual effects manifest at the group-level is severely lacking. This is a critical knowledge gap because many animal species rely on the ability to form cohesive groups that are essential to their feeding and mating biology, as well as for their capacity to avoid predators.

In this study, we examined how a multigenerational exposure to a pervasive antidepressant pollutant, fluoxetine, affects the structure, movement and foraging behaviour of guppy (*Poecilia reticulata*) schools. We tested mixed-sex schools of fish across two ecological contexts (in an open arena and in the presence of food) five times, both before and after exposure to one of three ecologically relevant exposure treatments (0 ng/L, 30ng/L, and 300 ng/L). We quantified repeatable group-level behaviours and outcomes, including group cohesion, alignment, leadership, and foraging efficiency using high-throughput animal tracking software. Since fluoxetine has previously been shown to increase activity and decrease sociality in fish, we predicted that fluoxetine exposed fish would exhibit weaker collective movement (e.g., reduced cohesion and alignment), resulting in a breakdown in group performance and consistency across contexts. I will present preliminary findings from this work, and show how behavioural tests in social isolation are insufficient for predicting the environmental risk of chemical pollutants in social species. I will also discuss robust methodological approaches for testing ecologically relevant behaviours across multiple levels of biological organization – from individuals to animal social groups.

#### 2.04.T-04 Linking Subtle Individual Responses to Population Level Effects of Pharmaceutical Exposure in an Outdoor Mesocosm

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Current environmental risk assessment of psychotropic drugs fails to reflect on the subtle effects that low drug concentrations

may have in aquatic environments. Due to the biologically active nature of psychotropics, their interaction with the nervous system, and their resemblance to infochemicals, they might affect behavior of organisms even at low environmental concentrations. At these low concentrations, effects on the individual survival might seem absent, but small changes in behavior can possibly result in changes at population and/or ecosystem level. To account for these subtle effects in the current environmental risk assessment, there is a need for new measurement endpoints to access effects across different levels of ecological organization (i.e., individual, population, community, and ecosystem). The main aim of this study is to find correlations between subtle behavioral effects and changes in the structure and functioning of an aquatic ecosystem as a result of exposure to low environmentally relevant concentrations of the antiepileptic drug carbamazepine.

The effects of chronic exposure to carbamazepine (14 weeks exposure to 0.001 – 100 µg/L) were studied in an outdoor mesocosm setup and combined with the behavioral analyses of two species. The locomotion patterns of *Gammarus pulex* and *Lymnaea stagnalis* individuals, taken from the mesocosms, were analyzed with a camera setup. Combined with population data of all species present in the mesocosms (macroinvertebrates and zooplankton), abiotic variables, and phytoplankton chlorophyll-*a*, this experiment obtained data that spanned three levels of biological organization: individual, population, and ecosystem level.

On community level we did not discover effects; the macroinvertebrate and zooplankton community composition were not affected by carbamazepine treatment. On individual level we recorded that locomotion of *G. pulex* had a strong photokinetic response to sudden darkness. The average swimming speeds dropped when organisms in the mesocosm were exposed to 1 µg/L carbamazepine.

Determining whether these individual behavioral endpoints are relevant at higher levels of ecological organization could emphasize the relevance of using behavioral endpoints for regulatory purposes. This experiment showed that the observed changes in average swimming speed of *G. pulex* do not propagate to community level effects.

#### **2.04.T-05 Networks in Aquatic Communities Collapse upon Neonicotinoid-Induced Stress**

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Neonicotinoids are the type of insecticides that are used most in the world are exceeding environmental norms in surface waters across the world. The actual risk to freshwater ecosystems remains unclear since translation from single species bioassays to actual community or ecosystem responses has proven notoriously difficult.

To this end, we investigated how the neonicotinoid insecticide thiacloprid potentially induces changes in invertebrate species composition and we linked individual species via the use of co-occurrence network analyses show how this degraded community coherence. By obtaining information on individual species feeding guilds and biomass, we were able to draw relations between species and their effects on ecosystem functioning.

We investigated the effects of the neonicotinoid thiacloprid on invertebrate species alpha, beta-diversity, co-occurrence, and ecosystem functioning using an outdoor mesocosm facility consisting of 36 individual ditches. These ditches are fully naturally colonized by biota. Here, we exposed the naturally formed freshwater ecosystem to an environmentally relevant concentrations of thiacloprid: 0, 0.1, 1 and 10 µg/L (n=9). We measured the aforementioned parameters following a before-after control design. By using feeding trait data and several measured ecosystem processes, we related individual species to their relative roles in the ecosystem.

We observed no effects on alpha diversity, while invertebrate thiacloprid concentrations induced a significant transition in invertebrate biodiversity. This coincided with a severe impairment of the co-occurrence network to such an extent that all thiacloprid impacted communities could not be statistically deviated from 1000 randomly generated communities (i.e., a null model) while the control significantly outperformed the null model, implying biological organization.

This degradation of community coherence coincided with significant reduction in the shredder feeding guild. In turn, this led to a stunted organic matter consumption and strong dominance of floating algal beds which suppressed phytoplankton growth (as modeled using piecewise structural equation modeling).

We found notable and strong effects below nearly all recognized toxicity levels for thiacloprid using a co-occurrence and feeding guild approach, showcasing the potential for these methods to couple species data to higher levels of biological organization.

#### **2.04.P Are the Sub-Individual Responses Translated Into Effects to the Higher Level of Biological Organization?**

##### **2.04.P-Tu048 Evaluation of Individual and Mixed Toxicity of Preservatives in Household Chemical Products using Zebrafish Embryo/Larvae**

**Cha Eun Park, Sujin Lee and Kyunghye Ji, Yongin University, Korea, Republic of (South)**

Isothiazolinones and parabens have been widely used as preservatives in a variety of household chemical products. Increasing evidence has demonstrated that these chemicals disrupt endocrine system in animals; however, limited information is available on the developmental toxicity and their underlying mechanisms in a combined exposure. In the present study, the effects of mixed



exposure to benzisothiazolinone (BIT), methylisothiazolinone (MIT), methylparaben (MP), and propylparaben (PP) on development and thyroid endocrine system were investigated using zebrafish embryo/larvae. Hatchability and larvae survival were significantly reduced in PP 0.3 µg/L, PP 30 µg/L, BIT+PP 0.3 µg/L, and BIT+PP 30 µg/L treatment groups. Malformation rates were significantly increased following exposure to PP, and the combined exposure of PP and BIT exhibited a greater effect on the abnormal development. The transcription of genes related to thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) was significantly upregulated in fish exposed to BIT, MIT, and BIT+PP exposure groups. Our observation indicates that isothiazolinones and parabens have the potential to affect thyroid hormone feedback circuit, and co-exposure to these two compounds could augment endocrine disruption. Further studies are needed to understand interaction of compounds found in consumer products.

#### **2.04.P-Tu049 A Holistic Approach to Determine Freshwater Mussel Response to Pharmaceuticals in Virginia, USA**

**Kathleen Mayer**, Tyler Allen and Austin D. Gray, *Department of Biological Sciences, Virginia Tech*

It is documented that various classes of pharmaceuticals, such as antidepressants and nonsteroidal anti-inflammatory drugs, are entering aquatic environments as co-occurring contaminants or mixtures. As a contaminant of emerging concern, there is a need to investigate the role of pharmaceutical pollution in biodiversity loss. Stream networks commonly receive pharmaceutical contaminants, and these habitats within North America house the most biodiverse mussel populations. Due to mussel populations residing in areas commonly polluted with pharmaceuticals, there is a research need to understand how these ecologically important organisms respond to different classes of pharmaceuticals. Transcriptomic studies that have been conducted for different bivalve species have suggested that variation exists in expressed genes that regulate innate immune response. While these studies provide a molecular approach to address bivalve response to environmental stimuli, the focus is typically on marine bivalve species rather than freshwater species. This leaves a gap for this project to explore pharmaceutical effects on freshwater mussels that are declining within the southeast region of the United States in Virginia. This dissertation proposal project will consist of laboratory and field-based assessments that will utilize molecular and ecological approaches, such as real-time PCR (i.e. polymerase chain reaction) of key immune response genes, luminescent-based assays of key antioxidants (e.g., glutathione), “omic” approaches such as proteomics utilizing LC/MS instrumentation, and macroinvertebrate assessments to address how mussels and their communities respond to pharmaceuticals. As pharmaceutical pollution gains more attention, a more holistic approach to how organisms respond to these agents of global change is crucial to better evaluate the ecosystem and community responses.

#### **2.04.P-Tu050 PFAS in the Marine Environments: Does Perfluorotetradecanoic Acid (PFTeDA) Affect Marine Benthic Invertebrates?**

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Per- and polyfluoroalkyl substances (PFAS) include a variety of synthetic chemicals that are present in most of the everyday products and industries. Due to their widespread occurrence, growing numbers, persistence in the natural environments, bioaccumulation potential and toxicity they are now one of the major threats in the aquatic environments. Due to the fact that purification treatment plants are not able to remove these substances, PFAS have been detected in various abiotic (water, sediments) and biotic (fauna, flora) matrices in both freshwater and marine environments, specifically in areas under stronger anthropogenic pressure. Moreover, little is known about the levels and composition of PFAS in the marine environments and their effects on nontarget marine fauna. Perfluorotetradecanoic acid (PFTeDA), belonging to PFAS family, has been found to affect male reproductive system in terrestrial vertebrates, but the knowledge on PFTeDA toxicity to marine organisms, and specifically benthic key species, is lacking. Therefore, the main purpose of this study was to assess the toxicity of this emerging and understudied PFAS using an experimental approach in which the blue mussels *Mytilus trossulus* collected in the Gulf of Gdańsk (Poland) were exposed to 30 and 300 ng/L of PFTeDA over a period of 10 days. To understand PFTeDA effects, an integrated multibiomarker approach based on markers characterising the activity of xenobiotic detoxification systems, oxidative stress response, general health status, and physiological and morphological abnormalities was used.

No mortality during the experiment was observed. On the tissue level, various progressive and regressive changes were found. In the respiratory system, significant increase in the presence of the tissue oedema and necrosis, infiltration of hemocytes, as well as deformities were observed in the exposed mussels when compared to those from the control. In the digestive system, atrophied digestive tubules of hepatopancreas were seen in over 57% and 87% of exposed mussels (30 and 300 ng/L, respectively). Also, the presence of cellular markers of the oxidative stress (superoxidase dismutase activity) in the tissues of PFTeDA-exposed mussels were found. Thus, the PFTeDA appears to mainly affect respiratory and digestive systems which may lead to energetic imbalance and weakening of exposed individuals.

#### **2.04.P-Tu051 Microplastic Ingestion in Juvenile Meagre – A Wide-Spectrum Biomarker Approach**

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The impact of microplastics (MP) in aquatic biota is vast. However, many experimental studies are based upon the use of MP

concentrations which do not reproduce naturally occurring ones, especially regarding micron-sized fractions. While such studies contribute to better understand the worst-case scenarios of MP impacts in organisms, the sublethal effects elicited at environmentally relevant concentrations are often overlooked. Therefore, understanding if field-measured MP concentrations could trigger early-warning signs of toxicity in fish is of vital importance. The aim of the present study was to assess the impact of polyethylene (PE) powder ingestion in juvenile meagre (*Argyrosomus regius*). Microplastics' polymers, their size, shape, and concentration were chosen according to data retrieved from water and fish samples, originated from semi-intensive aquacultures on the Portuguese coast. The present study consisted of exposing 35 *A. regius* juveniles to a concentration of 36 PE particles L<sup>-1</sup> for 21 days. Fish tissue (n = 18) was sampled for the biomarker analysis, namely: cortisol, digestive enzymes, antioxidant enzymes, respiratory enzymes, ubiquitin, heat-shock protein content, fatty acids, and carbohydrates content. The remaining 17 specimens were transferred to clean water and kept to depurate (7 days), to account for possible plastic excretion. After depuration, fish tissue was sampled for analysis of the above-mentioned biomarkers. Understanding the potential sublethal effects (i.e., inflammatory response) of PE ingestion is of utmost importance, once the detrimental effects to marine biota are still poorly understood and expected to increase as a result of ongoing accumulation of produced pollution and climate change, that rapidly alter environmental paradigms. Biomarker analysis is ongoing, as well as the determination of eventual particle degradation during exposure. Nonetheless, early results allow to expect inflammatory responses caused by accumulation or tissue damage by MP. As meagre is intended for human consumption, both environmental and human health matters can be at stake, which usually gains higher interest from decision-makers concerning pollution policy and regulation, to which this study intends to contribute to with further information.

#### **2.04.P-Tu052 Sublethal Effects of Thallium in the Freshwater Flatworm *Girardia tigrina***

**Patricia Caballero Carretero<sup>1</sup>, Diana Campos<sup>2</sup>, João L.T. Pestana<sup>2</sup> and Jose-Luis Martinez-Guitarte<sup>3</sup>,** (1)National Distance Education University (UNED), Spain, (2)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Aveiro, Portugal, (3)UNED, Spain

Thallium is a highly toxic metal present in the environment at low concentrations. Nowadays, it is considered an emergent pollutant because of its use in industries, metal mining, and smelting. Moreover, it is one of the main wastes from electronic trash. Due to these anthropogenic activities, it has been shown that thallium reaches freshwater ecosystems. However, only a few studies are assessing the toxicity of thallium in freshwater organisms at environmental concentrations, and the majority are focusing on *Daphnia magna*. This study assesses the effect of the exposure to thallium (I) nitrate in the freshwater flatworm *Girardia tigrina* at environmentally relevant concentrations. The aim of this study is to broaden the information of the thallium's effect in other freshwater organisms.

Planarians with a length of 1 cm were exposed to a range of sublethal concentrations of thallium nitrate (25 µg/L, 75 µg/L, 225 µg/L, 675 µg/L) for 8 days. In this study behavioral endpoints as locomotion and feeding rate but also head regeneration after decapitation were analyzed. The biochemical responses have also been analyzed measuring lipids, carbohydrates, and proteins contents and the oxygen consumption rate. Enzymatic activities like glutathione S-transferase (GST), catalase (CAT) and acetylcholinesterase (AChE), as well as lipid peroxidation (LPO) and total glutathione (tGSH) have been also analyzed to address effects on the antioxidant system of planarians.

Preliminary results show that planarians cannot regenerate after an exposure to concentration of 675 µg thallium /L and higher. However, sublethal concentrations do not elicit significant effects on locomotion or feeding rate. Regarding the biochemical response, planarians tend to decrease their consumption of energy reserves at the highest concentration studied. Finally, a decrease of GST and AChE activity is shown after an exposure of 75 µg/L as well as evidence of increased lipid peroxidation exposed to thallium. These data suggest that low concentrations of thallium disrupt detoxification and neurotransmission pathways in planarians. Results highlight the importance of molecular level effects of low concentrations of thallium with additional research at transcriptional level needed to have a global scenario of its ecotoxicity to planarians.

#### **2.04.P-Tu053 Fish Parasites as Bioindicators of Element Exposure in the Marine Environment: An Environmental Parasitology Approach**

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Anthropogenic activities, resulting in elemental exposure, in proximity to the marine environment reportedly have major impacts on ecosystem health and functioning. It is therefore important to consider all aspects of these ecosystems to better understand how ecosystems function. Parasites (endoparasitic worms) have been reported as promising sentinels for element accumulation; however, studies like these are lacking in South Africa. Linking cryptofauna to ecosystem health through environmental parasitology can possibly explain various accumulation patterns. Thus, the present study aimed to determine the elemental concentrations in the South African endemic fish, *Diplodus hottentotus* (Zebra) and its intestinal acanthocephalan parasites (also known as spiny- or thorny-headed worms). Fish were collected in the infratidal zone at the coastal town of Chintsa, along the east coast of South Africa. All five Zebras collected were infected with an acanthocephalan from the family Pomphorhynchidae with low (<30) to high (>30) intensity of infection. Fish were weighed, measured, and dissected, and collected muscle, liver, and parasite tissues were frozen for element analysis. A subsample of acanthocephalans was fixed for morphological and molecular identification. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to measure the element concentrations in host muscle and liver tissue, as well as the acanthocephalan parasite tissue. Statistical significance was determined using one-way ANOVA and Kruskal-Wallis tests. Essential elements and arsenic accumulated in significantly higher concentrations in the host

tissue compared to the parasites. In contrast nonessential elements (e.g., chromium, cadmium, lead) were significantly higher in the parasites than the host tissues. Although Al, Fe, and Zn were the highest elements measured, these do not reflect metal pollution but rather natural geogenic input into the nearshore marine environment. The high arsenic levels are derived from natural upwelling events in the region. The higher nonessential elements in the parasites reflect the inability to regulate the elements to the same degree as their hosts. These results underline the role that parasites play in ecosystem functioning such as reducing possibly toxic elements from accumulating in their host tissue.

#### **2.04.P-Tu054 Changes in the Ultrastructure of a Community of Microalgae and a Toxigenic Cyanobacterium Exposed to a Commercial Formulation of Glyphosate**

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Human activities influence the health of aquatic ecosystems, as many harmful chemical wastes are dumped into freshwater bodies. Intensive agriculture contributes to deterioration by discharging fertilizers and pesticides that affect the aquatic biota. Glyphosate is the most widely used herbicide in the world, and microalgae are particularly sensitive to its formulations.

Exposure to glyphosate can cause some green microalgae to be displaced from the phytoplankton community, altering the floristic composition that could favor the dominance of cyanobacteria. The combination of chemical stressors, such as glyphosate, and biological stressors, such as cyanotoxins and other secondary metabolites of cyanobacteria, could produce a combined and potentially more harmful effect on microalgae; these could affect the algal growth, physiology, and morphology. This study evaluated the combined effect of glyphosate (Faena®) and one toxigenic cyanobacterium on the morphology and ultrastructure of microalgae in an experimental community. For this, a strain of *Microcystis aeruginosa* (a cosmopolitan cyanobacterium that forms harmful blooms), and the microalgae *Ankistrodesmus falcatus*, *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, and *Scenedesmus incrassatulus*, were cultivated individually and together, exposing them to sub-inhibitory concentrations of glyphosate (IC<sub>10</sub>, IC<sub>20</sub>, and IC<sub>40</sub>). Effects were evaluated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Exposure to Faena® (glyphosate) affected microalgae's external morphology and ultrastructure, both individually and in combined cultures. SEM showed a loss of the typical shape and integrity of the cell wall and an increase in biovolume. In TEM, reduction and disorganization of the chloroplast, variation in starch and polyphosphate granules, formation of vesicles and vacuoles, degradation of the cytoplasm, and loss of continuity of the cell wall were observed. The combined exposure of microalgae to glyphosate and cyanobacteria produced more severe alterations than those described above. The presence of *M. aeruginosa* was an additional stress factor for the microalgae that added to the chemical stress produced by Faena® and increased the damage to the algal morphology and ultrastructure. These results warn about the effects of glyphosate and toxigenic cyanobacteria on algal phytoplankton in polluted and anthropically eutrophicated freshwater ecosystems.

#### **2.04.P-Tu055 Ultrastructure Changes in the Midgut of Stingless Bees *Melipona scutellaris* Exposed to Fungicide Pyraclostrobin**

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Stingless bees of the Meliponini tribe, *Melipona scutellaris* (Latreille, 1811), are native to the northeast region of Brazil. These native bee species hold economic importance and relevance related to the pollination of native plants. Fungicides have been widely used in agriculture, and forager bees can be exposed to them by a collection of floral resources (nectar and pollen) containing residues. Residual pesticide molecules in ingested food can be absorbed in the midgut of bees. In this context, the goal of the present study was to evaluate the effects of sublethal concentrations of pyraclostrobin on the midgut of *M. scutellaris* forager workers using an ultrastructural approach. Bees were collected from three nonparental healthy managed colonies (Rio Claro city, São Paulo State, Southeastern Brazil) and kept at laboratory conditions (28 ± 1°C, 70 ± 5% humidity) in cages. The caged bees were orally exposed for five days to two concentrations of pyraclostrobin in syrup (50% sucrose solution): 0.125 ng a.i./μL (P1) and 0.005 ng a.i./μL (P2). Control bees were fed a no-fungicide sucrose solution, and the acetone solvent control received a sucrose solution containing acetone (1% of the final volume). Each experimental group contained 80 bees (n = 20 per cage in four replicates). At the end of the toxicological bioassay, midguts from cooling-anesthetized bees (n = 3 per experimental group) were dissected and immersed in a formaldehyde-glutaraldehyde fixative (Karnovsky) for 2 hours at room temperature and routinely processed for Transmission Electron Microscopy. The ultrastructural analysis demonstrated that both fungicide concentrations induced alteration in the midgut, such as cytoplasmic vacuolization (more intense in P1), presence of atypical nuclei morphology, or pyknotic nuclei indicative of cell death (both were more intense in P1). Additionally, there was an alteration in the ultrastructure of spherocrystals (P1), which could be a reflex of cellular metabolism impairment and excretion of toxic products in this metabolism in the digestive cells as a response to fungicide exposure. The results of the present study reinforced the hypothesis that orally ingested pyraclostrobin induced cytotoxic effects in the midgut of native stingless bees. These cell responses of the midgut at the sub-individual level be a prelude to a reduced survival rate at an individual level, as observed in our previous study performed by our research group.

#### **2.04.P-Tu056 Cell Biomarkers and Behavioral Evaluation on the Neotropical Solitary Bee *Tetrapedia diversipes* Topically Exposed to Imidacloprid Insecticide and Pyraclostrobin Fungicide**

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Solitary bees present great species diversity, but ecotoxicological studies about them are still scarce. This study aimed to evaluate the effect of residual doses of imidacloprid and pyraclostrobin, alone and in combination, on the behavioral pattern (individual response) and the fat body (sub-individual response) of the neotropical solitary bee *Tetrapedia diversipes* (Klug, 1810). Males and females of newly emerged adults were submitted to bioassays of acute topical exposure. Experimental groups were assayed: control, solvent control, imidacloprid (0.0028 ng/μL), pyraclostrobin (2.7 ng/μL), and imidacloprid + pyraclostrobin (at the same concentration as pesticide isolated). Video tracking evaluation was performed with the treated adults along postexposure time (1 h, 24 h, 48 h, 72 h, 96 h) in a flat laboratory arena. The videos were analyzed using the EthoVision software to measure: velocity, moved distance, percentage of resting time, and percentage of mobile time. After 96 hours of acute contact exposure, each experimental group's fat body from females and males was dissected and processed for histological analysis. Histological slides were stained with hematoxylin and eosin (HE) for morphological analysis, and histochemical techniques: xylinidone ponceau (total protein detection) to determine the granulation stages of trophocytes; periodic Acid-Schiff (PAS) for cell polysaccharides detection. The data demonstrated that the residual doses applied in *T. diversipes* are sublethal. During the first 24 hours, the fungicide subtly reduced the distance and velocity of bees into the arena; the imidacloprid tended to increase the bee activity. Thus, at the individual level, both pesticides influenced the movement of bees, but the effects were minimized over time postexposure. Both oenocytes and trophocyte cells showed morphological and histochemical changes at the sub-individual level after topical pesticide exposure. Imidacloprid modified the protein granulation stage in trophocytes and pyraclostrobin increased the polysaccharides in these cells, which can lead to changes in the physiological processes dependent on protein and glycogen mobilization from fat body. This work is the first ecotoxicological study with a neotropical solitary bee species at a sub-individual scale and cellular level. The data obtained will broaden the knowledge concerning the effects of residual doses of imidacloprid and pyraclostrobin on native bees.

## 2.04.V Are the Sub-Individual Responses Translated Into Effects to the Higher Level of Biological Organization?

### 2.04.V-01 Comparison of Species Responses to Mixed Metal Exposure

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Humans and other animals are exposed to mixed chemicals present in water, air, or food. Mixed metal exposures are common in the aquatic environment, affecting both invertebrates and vertebrates. In the present study we determined the effects of metal mixtures in a time dependent concentration gradient from a lake receiving runoff water from a decommissioned mine. Acute toxicity was determined for four invertebrate models, *Caenorhabditis elegans*, *Daphnia magna*, *Ceriodaphnia dubia*, and *Heterocypris incongruens*, and one vertebrate model, Zebrafish (*Danio rerio*) and two cell lines, Zebrafish liver cell line (ZFL) and Human hepatocellular carcinoma cell line (HepG2). The metal mixes elicited the highest mortality in *D. magna* (100%), followed by *C. dubia* (100%), *H. incongruens* (95%), ZFL cells (65%), *D. rerio* (37%), human HepG2 cells (20.5%) and with the lowest response observed for *C. elegans* (7%). Analysis of gene expression showed that the metal response genes, mt-1 and mt-2 correlated well to the exposure conditions in all tested models. The results show that *D. magna* is the most sensitive model organism for effect identification of mixed metal exposures. In such exposures, significant upregulation of metallothionein genes was protective to *C. elegans*, *D. magna*, and HepG2 cells.

## 2.05 Arthropods at Risk? Current and Future Perspective on Insect Ecotoxicology

### 2.05.T-01 Effects of Clothianidin and Flupyradifurone on Bumblebee Colony Development at Fluctuating Ambient Temperature

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Although neonicotinoids and the replacement flupyradifurone share the same mode of action, the latter is marketed as pollinator-friendly. Knowledge of their effects on pollinators other than the Western honeybee is limited, and most focus has been on individual adult bees. Furthermore, few studies consider how environmental conditions such as temperature affect the toxicity of chemicals, especially under chronic exposure. Lastly, risk assessment is performed on one chemical at a time, while mixture toxicity is a more relevant scenario for bees.

We study how the neonicotinoid clothianidin and flupyradifurone, alone and in combination, affect colony development of the buff-tailed bumblebee (*Bombus terrestris audax*) under fluctuating ambient temperature. We expected colder temperatures and higher concentrations or mixture treatments to elicit stronger adverse effects. Bumblebee colonies were exposed to one of six treatments: a low (4 mg/L) or a high (4 x low) concentration of clothianidin, a low (1 mg/L) or high (4 x low) concentration of

flupyradifurone, a mixture of the two at low concentration or a control treatment. Exposure was through artificial nectar over 21 days, followed by 21 days postexposure. The experiment ran sequentially from May until August to allow a natural ambient temperature gradient.

The effects of temperature on bumblebee colony development depended on the focal endpoint, insecticide, and concentration, and no general temperature-dependent toxicity was identified. Only exposure to the high concentration of flupyradifurone followed the expectation of more adverse effects at colder temperatures when looking at weight gain and the number of bees in the colony at the end of the experiment. Low exposure concentrations of flupyradifurone were more toxic at warmer than colder temperatures for both endpoints. The low concentration of clothianidin showed a complex interaction with temperature, while the high concentration of clothianidin was more toxic at warmer temperatures. The mixture treatment had comparable effects to flupyradifurone for the number of bees and gave a stronger negative effect on weight gain than when the insecticides were administered alone.

This study shows the importance of testing field-realistic doses over a realistic exposure period in combination with natural variables. Climatic conditions and multiple stressors should be considered when performing risk assessment of chemicals.

#### **2.05.T-02 Comprehensive Lipid Profiling to Study the Effect of the Growth Inhibitor Insecticide Teflubenzuron on the Nontarget species *Folsomia candida* over time**

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The application of pesticides affecting the growth of invertebrates raised concerns about their impact on nontarget species, as most of these chemicals end up in the environment. Although classical risk assessment methods can provide valuable information about the toxicity of chemicals, they cannot uncover the affected molecular pathways. Omics-based approaches in combination with phenotypic assessment can provide insights into potential biomarkers and most affected pathways in animals exposed to chemicals. This study aimed to investigate the lipidome of springtails *Folsomia candida* exposed to environmentally relevant concentrations of the insecticide teflubenzuron (0.000, 0.006, 0.014, and 0.035 mg/kg of soil) over time. To characterize the lipidome of *F. candida*, the time points as 1, 2, 7, 14, and 21 days were selected. The results showed clear separation in the lipidome of *F. candida* on different days, as the lipidome was affected by the reproduction cycle of springtails and the presence of offspring. Considering that all time points were significantly different, the response to teflubenzuron can be expected to be different through the exposure duration. The focus was on days 7 (only adults present in the soil) and 14 (adults inhabit together with the first generation of offspring). In both groups, the decrease in N-acyl ethanolamines and phosphatidylcholines is an indicator of membrane disbalance and oxidative stress. On day 7, upregulation of Omega-3 fatty acids, carnitines and ceramides was observed. This group of lipids plays an essential role in fatty acid  $\beta$ -oxidation in mitochondria. Down-regulation of triacylglycerols (TG) and diacylglycerols (DG) indicates effects on lipid storage, cell signaling, and energy metabolism alterations. Exposed groups from day 14 showed also the regulation of phosphatidylglycerol. This class is exclusively present in the inner membranes of mitochondria and plays a key role in the defense mechanism and cell apoptosis. The results of this study indicated the importance of following molecular disruption during different life stages to determine the possible pathways affected by chemicals. Additionally, analysis of springtails exposed to teflubenzuron showed a significant effect on lipid homeostasis and storage, membrane synthesis, and energy metabolism. Moreover, the current results suggest that insecticide causes inflammation and oxidative stress.

#### **2.05.T-03 Exploring Plasticisers Mix Physiological Impacts on the Moth Larvae *Spodoptera littoralis* (Noctuidae)**

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The production of plastics and plasticisers has greatly increased in the recent decades, along with uncontrolled plastic discharges. Accumulation of plastic debris in the environment, and the progressive release of their main constituents, in particular the plasticisers Bisphenol A (BPA) and Di(2-ethylhexyl)phthalate (DEHP) have increased pollution intensity in many terrestrial and aquatic habitats. As plasticisers can act as Endocrine Disrupting Chemicals (EDCs), it is crucial to increase our understanding of their potential effects on wildlife, including terrestrial invertebrates that have been less examined than their aquatic counterparts.

So far, we previously demonstrated the deleterious impacts of these two plasticisers on a terrestrial insect, the pest *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) fed on artificial diet containing DEHP or BPA. To mimic environmentally realistic exposures of these two compounds, we developed a new contamination approach by growing plants which were sprayed with DEHP, BPA and mixture of the two compounds. Larvae of *S. littoralis* were further fed with leaves from these plants, thus allowing to observe if realistic contamination also alter postembryonic development. We then completed previous metabolic analysis showing energetic disruption by the development of a glycolytic enzyme activities that would help us to better understand the DEHP metabolic disruption, when larvae are fed by laboratory food.

Experiments showed that postembryonic development disruption could be observed in *S. littoralis* when exposed to contaminated tomato leaves at larval stage. Our results emphasized the need to study co-occurrent EDCs on terrestrial invertebrates into trophic chain. We also found that individuals could be affected differently according to their sex. This parameter should be considered in

future studies working on EDCs effects. Some mechanisms underlying the disruption in larval stage were explored by the determination of glycolytic enzyme activities. More mechanisms have to be explored to explain postembryonic development disruption. Next studies should focus on hormonal potential disruptions for the co-occurrence of EDCs in larval stage. BPA, DEHP, and its metabolites could be quantified in the future in tomato leaves and larvae of *S. littoralis*. Finally, it would be also interesting to get more information on other levels of the organism, such as gene expression and metabolomic profiles.

#### **2.05.T-04 Practical Implementation of the Assessment of Invertebrate Behavior and Its Use in Behavioral Ecotoxicology**

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Sublethal behavioral endpoints as a tool for modern ecotoxicology become a promising element at the interface among basic sciences and regulatory practices. So far, the lack of standardization of behavioral test methods makes them rarely considered in a regulatory context, even though they show enhanced sensitivity compared to standard endpoints and can serve as early warnings. Our approach aims to achieve standardization by using high-throughput behavioral observations allowing us to quantify behavioral changes with an optical behavior monitoring tool. Behavioral abnormalities can have severe consequences on the individual and population levels. For example, disturbed locomotion activity can impact drift, mate-finding, predation risk, and thus the overall population dynamics. Chemicals never act alone in the environment, but rather in a framework of diverse other stressors, including natural ones, such as parasites and invasive species – which makes behavioral studies even more multifaceted. To quantify and compare behavioral responses we chose four local amphipod species (*Gammarus fossarum*, *G. pulex*, *G. roeselii*, and *Dikerogammarus villosus*) with different historical (e.g., native, invasive), but also ecological (e.g., healthy and parasitized) backgrounds. Common parasites are Acanthocephala which are known to manipulate their hosts' behavior. They accumulate pollutants and are suspected to influence the pollutant metabolism of the hosts. We exposed the amphipods to various anthropogenic chemicals, like active pharmaceutical ingredients (API) and insecticides. We then extended this to include the natural behavior-influencing stressor of parasitization. Our results revealed behavioral abnormalities within short time exposure (90-120 min) at sublethal and environmentally relevant concentrations of anthropogenic chemicals. Furthermore, our results show that behavioral responses seem to be species specific, with sensitive headwater species showing the strongest behavioral responses. Irrespective of chemical contamination, parasitization with acanthocephalans has resulted in generally higher activity of amphipods. Our approach shows the enormous potential of behavioral ecotoxicology and the realistic possibility of achieving standardization. With the right tools, it is feasible to create a comprehensive, interdisciplinary, multiscale approach to systematically identify behavioral endpoints and implement them in risk assessment of substances.

#### **2.05.T-05 In-Field Deposition of a Sprayed Reference Chemical on Flying Insects: Normalization of Overspray by Surface Area or Habitat?**

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There is growing concern in society about insect biodiversity decline, and the potential impact of agrochemicals. The risk assessment procedures of agrochemicals for nontarget arthropods (NTA), pollinators, and other terrestrial invertebrates is aimed to be a conservative evaluation of exposure calculations and effect studies, both in the field and the near off-field area. The data requirements for NTA risk assessment in the EU consist of effect studies with at least two standard NTA organisms exposed only to dried residues, using glass plates in tier 1 or on leaf material and quartz sand in extended lab studies. The spray dose applied in these relates to recommended product use (in g a.s./ha), allowing for a risk analysis based on applied field rate and lethal/effect rate in the experiment. In a field where chemicals are sprayed on crops, the NTA present above the soil are exposed via direct overspray, via contact with (fresh and) dried residues on plant material and soil surface material, and via intake from residues accumulated in food (plant material, prey, soil surface material). The risk assessment for bees and other pollinators already includes data requirements on effects by overspray (droplet contact test), and intake via food (via spiked feeding solution). In the current study, we focus on the in-field risk to NTA and pollinators due to direct overspray. The current study aimed to better characterize the actual deposit on NTA and non-managed pollinators (e.g., 90-percentiles per species), compared to the applied dose rate. A first field study established the vertical range of spray deposits of a fluorescent reference chemical using filters fixed at five different heights in a field of densely planted (nonflowering) White mustard (*Sinapis alba*). A second field study determined the spray deposit of the fluorescent tracer on NTA and pollinators present in the top layer of in a flowering field of oilseed rape (OSR). The deposit per surface area of individual organisms (or pooled samples of one species) was measured and compared to the deposit on filter discs placed horizontally in the OSR top layer. Collection of organisms was done by collecting bumble bees and solitary bees at nesting boxes, net sampling, hand-catching of individuals within two hours after spraying. Various flying insects have a 90-pct deposit per surface area that is 37-86% of the disc filters, deposits on bugs range between 4-30% of the applied rate.

#### **2.05.P Arthropods at Risk? Current and Future Perspective on Insect Ecotoxicology**

## **2.05.P-Tu057 A Laboratory Test for Assessing Effects of Plant Protection Products on the Herbivorous Nontarget Arthropod *Locusta migratoria* (Caelifera, Acrididae)**

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A newly developed laboratory test with nymphs of the herbivorous locust *Locusta migratoria* as surrogate species for oral toxicity to nontarget arthropods is presented. Grasshoppers are abundant in agricultural landscapes, and therefore may represent a relevant group of arthropods for testing oral toxicity, which was considered a relevant exposure route in the 2015 EFSA Scientific Opinion on non-target arthropods. The objective of the test is to assess the oral toxicity of pesticides to locust nymphs including sublethal effects on the reproductive capacity of the developed adults. Seven days old nymphs are exposed to treated maize leaf discs for 48 hours in the first phase with mortality assessments after 24 and 48 hours. At the end of the exposure period, the amount of consumed leaf area is quantified by video analysis to confirm sufficient exposure. In the second phase, the surviving locusts of each treatment group are kept in one rearing cage for approx. seven weeks until adult stage and sexual maturity is reached. During this time, mortality of different developmental stages is recorded at regular intervals. Adult male and female locusts are allowed to mate in the same rearing cages. For egg laying, one oviposition cylinder per cage filled with wet coconut humus is offered through an opening in the bottom of the cage. Oviposition cylinders are renewed after 48 hours to count eggs in two consecutive samplings. The egg pods are sieved out and incubated in a climate cabinet for three to four weeks. Bellaplast bowls equipped with mesh cover and filled with wet coconut humus are used as egg pod containers. After day 10, the bowls are daily checked for hatched nymphs until no further hatching occurs for seven days, and egg pods are dissected for counting unhatched eggs. The total test duration is 10 to 12 weeks.

The endpoints of the test are the nymphal mortality after 48 hours, the cumulative mortality recorded for different developmental stages until adult maturity, the mean number of eggs per surviving female per day and the proportion of fertile eggs: The test is carried out in a dose-response or limit design. Water and toxic reference (e.g., dimethoate) test groups are used as negative and positive controls. First results of a dose-response design study with dimethoate are presented.

Future work shall focus on the determination of reliable validity criteria and optimisation of the test design.

## **2.05.P-Tu058 Development of an Ecotoxicological Testing Method to Assess Side-Effects of Plant Protection Products on Herbivorous Lepidopteran Larvae (*Spodoptera exigua*) under Laboratory Conditions**

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In the current environmental risk assessment of Plant Protection Products (PPP) to nontarget arthropods (ESCORT 2), oral exposure via food ingestion by herbivorous species is not considered and therefore, no standard laboratory test protocols for oral exposure of arthropods other than bees are available. In the 2015 EFSA Scientific Opinion on nontarget arthropods, Lepidoptera is one of the suggested taxa to cover this herbivorous oral route of exposure. The plant-feeding caterpillars can easily consume contaminated plant material while the adults are often nectar feeder and pollinators, and the taxon therefore plays an important role in many ecosystems of Europe.

Studies performed over the previous years showed that this approach is feasible in general. However, more work is needed to see if it is possible to develop a robust and seasonally independent testing method. For this purpose, in May 2022 CropLife Europe NTA expert subgroup initiated the development of an ecotoxicological testing method to assess effects of plant protection products on herbivorous lepidopteran larvae of *Spodoptera exigua* under laboratory conditions.

Suitability and challenges of using *Spodoptera exigua* as herbivorous species for ecotoxicological testing and the relevance for the risk assessment have been investigated.

The objective of this poster is to create awareness and to give an overview of this ongoing project. In particular the main challenges and possible solutions will be presented and discussed in order to obtain a broadly accepted testing method.

The research was divided into two parts (a) finding the best handling and food for *Spodoptera exigua* prior and after to the exposure phase and (b) the assessment of mortality, food consumption and sublethal endpoints of this herbivorous lepidopteran species after exposure to a reference item.

## **2.05.P-Tu059 DEHP and BPA Act as Endocrine Disrupting Chemicals in the Pest Moth *Spodoptera littoralis***

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Bis(2-ethylhexyl) phthalate (DEHP) and Bisphenol A (BPA) are a widely produced plasticiser found in many everyday life products like medical tools and floor covering. DEHP and BPA are found everywhere in the environment, in particular in sediments, soils, and plants. They are recognised as an endocrine disrupting chemical (EDC) in vertebrates. While invertebrates represent most of animal biodiversity and play critical roles in the aquatic and terrestrial ecosystems, few studies have focussed on the effects of these plasticisers on those species and particularly in terrestrial insects whereas the two lipidic hormone families,

ecdysteroids and juvenile hormones, are involved in the control of the postembryonic development and adult physiology in insects.

In this context, we used the Egyptian Cotton leafworm *Spodoptera littoralis* as a model to study the effects of larval exposure to DEHP and BPA on postembryonic development, male sexual behaviour, and development of the offspring of treated males or females. We showed that, as in vertebrates, these two compounds act as EDC in this species by affecting the ecdysteroid pathway, and alters postembryonic development, male sexual behaviour, and life-stage duration in the offsprings.

#### **2.05.P-Tu060 Collembola in Depth: Temporal Variability of Vertical Stratification in the Soil Profile**

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Springtails (Collembola) are among the most abundant groups of soil decomposers, inhabiting various organic substrates and using a wide range of food sources. They show a clear vertical stratification, with some taxa occurring in the soil only (euedaphic species) to taxa living partly within the soil and partly on the surface (hemiedaphic) to species living mainly on or above the ground (epedaphic). Depending on substrate quality and abiotic conditions, springtails move between these layers and resulting differences or changes in vertical stratification in the springtail community composition can be substantial. While the spatial aspect of this phenomenon is well known, the temporal variation in this vertical stratification is not documented so far.

Using aspirator and stratified activity-based in-soil sampling ("mine traps") in an agricultural field, we investigate the occurrence and abundance of springtail species on the ground (aspirator sampling) and in different soil depths (-10 cm, -20 cm, mine traps). Data were collected over more than two years, covering two cropping systems (sugar beet, winter wheat). We investigated the effect of habitat quality (crop identity, BBCH) and abiotic conditions (rain, temperature, season) on the vertical stratification of springtails and examined the temporal variation in these ecological profiles.

Here we ask: (1) Can we observe species specific vertical shifts over time (upward or downward), and can we link these shifts to environmental conditions (crop, abiotic conditions)? (2) Do (differences in) species-specific vertical shifts lead to altered soil layer-specific springtail communities?

Changes in soil microclimate might lead to new soil invertebrate community structures, where both overall abundances and layer-specific species composition could change. Such a shift in vertical distribution or vertical stratification of soil springtails in response to environmental conditions can have large effects on the soil food web and other soil processes.

#### **2.05.P-Tu061 Effects of Insecticides at Different Levels of Biological Organization: Using Changes in Lipidomics to Explain Effects on Growth and Reproduction of *Folsomia Candida* Exposed to Teflubenzuron**

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Standard methodologies to assess the effect of pesticides on Collembola lack a mechanistic understanding of the changes in reproduction, growth and survival, hence fitness. The use of untargeted metabolomics in risk assessment can uncover the molecular pathways leading to adverse outcomes. Untargeted screening of molecular changes over time (such as changes in lipidome) provides a more detailed picture of the sublethal effects of pollutants present in the environment. In this study, we determined the effect of the insecticide teflubenzuron on the lipidome, growth (biomass) and reproduction of *Folsomia candida* (22 days old) exposed to 0.000, 0.006, 0.014, and 0.035 mg a.s kg<sup>-1</sup> LUFA 2.2 soil. The endpoints were measured after 1, 2, 7, 14, and 21 days of exposure.

The results showed a trade-off between growth and reproduction of *F. candida* at the highest concentration tested. The number of offspring produced on day 21 differed significantly from the control, but the growth of the adults was not affected. Significant changes in reproduction on day 21 suggested the inhibition of egg laying between days 5 to 10 of exposure. Therefore, the lipidome of the adults on day 7 was assessed in order to link lipid alteration with growth and reproduction. The main affected lipid classes were N-acyl ethanolamines (NAE), fatty acids (FA), diacylglycerols (DG), and triglycerols (TG). The increase of NAE and FA clearly indicates inflammatory reaction, lipid peroxidation, and energy metabolism disbalance, which can explain the decrease in reproduction. Overall, TG and DG play a vital role in egg production, therefore, their decrease can be early indicators of reproduction deprivation caused by teflubenzuron. Additionally, phospholipids (PC) did not change in exposed animals which corresponds to the lack of changes in growth, since PC are the main contributors to the cellular membrane.

The results of this study for the first time use changes observed in the lipidome of *F. candida* to explain effects on individual fitness. The trade-off between a decreasing number of offspring and continuous growth was supported by the outcomes of the lipidome assessment. Dysregulation of the lipid classes as TG, DG, FA, and NAE suggests deprivation in reproduction, possible starvation, and inflammatory reaction to exposure. Further, combined studies including different species can contribute to a better understanding of the toxicity of teflubenzuron from the molecular to the population level.



## **2.05.P-Tu062 Uncovering Potential Chemical Stressors for Insects: *In vitro* Toxicity of Soil and Water Samples from Private Gardens**

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Insects as a large taxonomic group are experiencing a drastic biodiversity decline, while covering many niches in ecosystems and providing important functions. Therefore, it is of great concern to further uncover causes, with the goal of identifying mechanisms to dampen species loss. The interdisciplinary project “SLInBio” ([insektenvielfalt-frankfurt.org/](http://insektenvielfalt-frankfurt.org/)) is investigating how the perception and valorization of insects can be increased in Frankfurt am Main and what contribution cities can make to the conservation of insect diversity. Hence, social-ecological interactions between lifestyles and everyday practices of city dwellers and urban insect diversity are being analyzed. This includes a strong communication with garden owners, since they hold the potential to influence small-scale habitat quality: in urban areas, private gardens display a relevant amount of the total green area distributed among the city; hence gardens pose important habitats for organisms. But individual practices and preferences in aesthetics and the usage of pesticides or fertilizers, chemical run-off from building materials, water management or animal keeping can strongly influence habitat quality and thus insect biodiversity. As an ecotoxicological approach, soil and water samples were collected from 17 gardens and their ponds, distributed among the city of Frankfurt am Main. Samples were extracted and tested with toxicological *in vitro* assays to determine potential toxic effects. Baseline toxicity was assessed with the Microtox® assay, mutagenicity with an Ames test and dioxin-like activity with a yeast reporter gene assay. The results revealed significant differences between the sampled gardens regarding chemical pollution by biologically interfering substances. Gardens are classified depending on the practices that gardeners apply in their area, which were identified by a survey performed by the Institute for Social-Ecological Research Frankfurt am Main. The survey results will then be connected to the ecotoxicological data. This will identify the impact that individuals have on toxicological parameters relevant to aquatic and terrestrial organisms, hopefully uncovering feasible changes that can be performed by gardeners on their private property to support local insect diversity.

## **2.05.P-Tu063 Critical Review and Recommendations to Improve the Quality and Reproducibility of the US EPA Chronic Mysid Testing Guideline: Update on a CropLife America and CropLife Europe Project**

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The US Environmental Protection Agency (EPA) OPPTS 850.1350 Mysid Chronic Toxicity Test (draft, 1996) is conditionally required (40 CFR Part 158.630) for the registration of pesticide products in the U.S. though this study is not required by regulatory agencies outside of North America (EU, for example), results have an impact on product registrations across the globe if the endpoint drives the risk assessment.

The primary challenges with successful completion of this study are due to a lack of detailed information within the EPA test guideline (TG) on the study design and conduct. Issues with test organism performance often result in studies that do not meet acceptability criteria, necessitating a study repeat.

Crop Life America (CLA) has previously identified issues concerning the language in the existing TG, including: 1) lack of clarity about study endpoints; 2) limited guidance for handling the second generation (G2) populations; 3) difficult to achieve data quality objectives for growth and reproduction; and 4) a need to update procedures to reflect methodological improvements following 20+ years of laboratory experience in performing this study. A joint CLA-CLE (Crop Life Europe) project was undertaken to review issues with study design and subsequently develop recommendations for the modernization of the TG. In addition, the relevance of using endpoints from marine organisms for an edge of field aquatic risk assessment is questioned and discussed.

## **2.05.P-Tu064 Bti-Induced Changes in Aquatic Subsidy Dynamics Transfer to Linked Terrestrial Food Web**

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Emerging aquatic insects link aquatic and adjacent terrestrial food webs and serve as high-quality prey in both habitats. The availability of this aquatic subsidy is of high importance for terrestrial predators such as riparian spiders, bats, or birds. In a previous study on aquatic subsidy conducted in twelve floodplain pond mesocosms (FPMs), a change in the emergence dynamics of the main constituent, (i.e., nonbiting midges (Chironomidae), was detected and attributed to the application of the mosquito control agent *Bacillus thuringiensis* var. *israelensis* (Bti). The resulting temporal reduction in prey density might induce the predators to switch to alternative, potentially less nutritious food sources. To investigate this, we collected female spiders (*Tetragnatha extensa*) from the riparian vegetation of the FPMs in parallel to the first study as well as their potential aquatic and terrestrial prey. We used stable isotope ratios to analyse the spiders' diet in terms of aquatic proportion, niche size, and trophic position. Additionally, the energetic budget (i.e., protein, lipid, glycogen, and carbohydrate content) of the Chironomidae was measured to track Bti-induced changes in their quality. While the content of the energetic components and thus the prey quality

did not significantly change, effects of Bti on the spiders' diet were observed. The trophic position was lower in treated FPMs while the aquatic proportion was slightly reduced. We assume that spiders did not only feed more on terrestrial prey but also on other aquatic organisms, such as mayflies, whose emergence was unaffected by Bti. These results show that the previously observed change in the emergence dynamics of Chironomidae can transfer adverse effects of an anthropogenic stressor from aquatic to terrestrial systems. It highlights that not only changes in the total abundance of aquatic subsidy but also temporal changes are of high importance. Terrestrial predators may suffer from a less nutritious diet during periods of increased energy demand (e.g., during reproduction or before hibernation) leading to reduced reproductive success or lower general fitness. Thus, we suggest on the one hand to include a temporal perspective in studies on aquatic-terrestrial linkages and on the other hand to intensify the investigation of cross-boundary effects of anthropogenic stressors.

#### 2.05.P-Tu065 Assessing Risk to Pest Control and Pollination: Which are the Representative Bulnerable NTA Taxa?

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Nontarget arthropods (NTAs), defined as arthropods living on the surface of soil or plants, are taxonomically diverse, exhibit a diverse range of traits and undertake many important ecological functions that support wider biodiversity and deliver ecosystem services (ES), in particular pest control and pollination. Identifying representative and vulnerable taxa that drive ES delivery will enable risk assessment to be more focussed and ecologically relevant. Here we present an approach for identifying representative vulnerable NTA in European agricultural landscapes. We focus on the ES of pest control by predators and parasitoids, and pollination (excluding managed bees), and six major arable crops: wheat, maize, barley, oilseed rape, potato, and beet. Using the arthropod database compiled by Riedel et al. (2016) and information in the EFSA scientific opinion on NTAs (EFSA 2015) we have identified representative NTA families for each ES by crop and by EU zone based on their abundance, dominance, diversity, distribution prior prioritisation. Vulnerability of representative NTA families was assessed following the method of de Lange et al. (2012). Key NTAs for pest control include: Linyphiidae (sheet weavers spiders), Lycosidae (wolf spiders) Carabidae (ground beetles), Staphylinidae (rove beetles), Coccinellidae (ladybirds), Syrphidae (hoverflies), Tachinidae (tachinid flies), Braconidae, Aphelinidae (parasitoid wasps) and Ichneumonidae (ichneumonid wasp). Key NTAs for pollination include: Andrenidae (mining bees), Apidae and Halictidae (bees), Noctuidae (owl moths), and Syrphidae (hoverflies). This information will help to develop a methodology for linking direct toxic effects and impact on ES and support the dialogue between risk assessors and risk managers by providing the scientific evidence necessary for the definition of specific protection goals for key ES provided by NTAs.

#### 2.05.P-Tu066 Effects of the Experimental Exposure to Triazole Fungicides on Two Groups of Insects

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The use of pesticides in agriculture is one of the main causes of biodiversity loss in agro-ecosystems. Triazoles fungicides are among the most widely used pesticide groups nowadays. The mechanism of action of these substances consists of impeding the formation of the fungal wall by inhibiting the formation of ergosterol, one of the wall's main components. Studies in vertebrates have reported that triazole fungicides may act in a similar way as in target fungi, affecting sterol synthesis and altering the levels of some sterols like the sexual hormones, which ultimately reduces the reproductive output. In arthropods, the alteration of the sterol biosynthesis pathway by triazoles may be interfering with the production of ecdysteroids, a type of hormones involved in moulting and reproduction processes. Invertebrates are key elements in ecosystem functioning because they maintain plant biodiversity as pollinators and serve as food base for other animals, hence it is necessary to understand how the use of triazoles in crops may be affecting them. We present two experimental studies to evaluate the effect of triazoles on two model species with different metamorphic cycles: an orthopteran, the Jamaican field cricket (*Gryllus assimilis*), with a direct development cycle, and a coleopteran, the yellow mealworm beetle (*Tenebrio molitor*), with a more complex metamorphosis. These two species are exposed to prothioconazole and tebuconazole formulations simulating field exposure scenarios either through food (simulating an exposure to seed coating products) or by overspray (simulating an exposure to foliar treatment). We monitor fertility, fecundity, alterations in the developmental rate, and malformations associated with the exposure to triazoles. Furthermore, we use HPLC-MS/MS to quantify ecdysteroid levels and analyse triazole residues at different life stages of the individuals. With this work we aim to disentangle how triazoles might be affecting arthropods as well as to characterise biomarkers of triazole effects on these animals in the field.

#### 2.05.P-Tu067 The Mealworm Dietary Exposure Lifecycle Test: A Potential Model System in Environmental Risk Assessments

**Michael-Thomas Ramsey**, Ecotoxicology, Labcorp Early Development, United Kingdom

This study was undertaken to explore the necessary procedures required for conducting a higher-tiered Good Laboratory Practice (GLP) Laboratory test to evaluate the effects of a test substance in a diet on the survival and reproduction of the mealworm, *Tenebrio molitor*.

*Tenebrio molitor* is a holometabolic insect that lives in flour, bran, and similar products. They are being recognised more as a beneficial insect as, economically, they are among the most important species used for the large-scale conversion of plant biomass

into protein. They also possess the ability to break down plastic with no ill effects or accumulation of toxic substances. They are a robust species that can thrive under laboratory conditions, which make them an ideal candidate system for EIA testing.

In this work, we showcase the four experimental trials from which we successfully determined the final protocol for this test design. Each trial was conducted to determine: (1) the time taken to complete study, (2) the viability of larva in diet (test substrate), (3) the most appropriate assessment techniques, (4) time duration of each life cycle stage, (5) a procedure for egg collection and assessment, (6) the acceptable control performance criteria across each life cycle stage, (7) the rate and reference item to be used for a toxic reference, and (8) the sample size required at each rate to accurately determine the lethal concentration for mortality and Effect Concentration for the reproductive output following exposure to a test item in the substrate.

We conclude this work with the results of a full test-run of the final protocol (water control and toxic reference only) that incorporated the optimised parameters determined from the four experimental trials. The exciting results obtained from this final experiment allow us to demonstrate the effectiveness of this test design and the potential of *Tenebrio molitor* as a test system.

## **2.05.P-Tu068 Refined Parameters for Bumblebee Microcolony Dietary Exposure Tests**

**Michael-Thomas Ramsey**, *Ecotoxicology, Labcorp Early Development, United Kingdom*

Honeybees have long been a surrogate for other non-*Apis* bees in laboratory testing owing to the vast understanding of their biology and their compliance with a laboratory setting. Recognising the importance of bumblebees to managed and natural landscapes, there is a pronounced need for bumble bee-specific methodologies to develop a better understanding of their biology and how these bees respond to various stressors. Current testing formats reduce experimental complexity, as they limit investigations to acute exposures in adult bees, and ignore important effects related to colony health and production of new progeny. As a result, microcolony studies have grown in popularity.

In this preliminary study, we explore the necessary procedures and parameters required to optimise a GLP laboratory microcolony feeding test to determine whether sugar syrup and pollen contaminated with doses of a test item could affect the food consumption, worker survival and the size of the brood, as well as cause any delay of adult emergence in the bumblebee *Bombus terrestris*.

In this work we showcase the results of the 5 individual trials that led us to the optimised parameters and processes for the final study design. These trials consisted of an 85-day study concept trial, a 35-day feeding syrup trial, a 42-day toxic reference trial, a 21-day revised enclosure feasibility trial and a 42-day dietary improvement trial.

Over these individual trials, conducted with the aim of minimising untreated control mortality, we successfully established the following: (1) the number of mother colonies required to produce the required amount of queenless microcolonies, (2) the most appropriate dietary sugar solution composition, (3) the frequency at which the micro-colonies require feeding, (4) the average time duration for worker-dominance and drone laying, (5) the natural mortality of individuals kept in this setting, (6) the most effective method for diet administration, (7) the optimal nest box design and the level of intervention required to maintain hygiene levels, (8) the sample size required at each rate to accurately determine the lethal concentration for mortality and Effect Concentration for the reproductive output following exposure to a test item in each diet.

## **2.05.P-Tu069 Longevity of *Apis mellifera carnica* and *Bombus terrestris* Exposed to Pesticides (Alone and in Combination) in Laboratory Conditions**

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The disappearance of honeybees and colony mortality have beset beekeepers worldwide in recent years. In that same sense, bumblebees have been shown to reduce occurrence and richness globally. Currently, pesticides and other environmental stressors have been linked to this issue. The pesticides are widely used in crops where bees forage and collect resources with residues that can harm their colonies. From this point of view, the present purpose was to evaluate and compare the effects of the fungicide Folicur EW 250 and insecticide Daskor 440, alone and in combination, on Carniolan honeybee (*Apis mellifera carnica*) workers and bumblebees (*Bombus terrestris*) longevity. The Carniolan honeybees were collected from three different colonies by the apiary installed at the University of Maribor, Hoče - Slovenia. The bumblebees were collected in lavender crops around the same place mentioned above. Both bee species were caged in plastic cups and transferred to an incubator at 28°C, with 70% relative humidity. Toxicological bioassays of oral exposure were performed. The foragers of both species were divided ( $n = 20$  bees in five replicates for Carniolan honeybee;  $n = 10$  bees per treatment for bumblebee) into three experimental treatment groups and control: fungicide Folicur EW 250 (0.250 ng/μL) - FGD, insecticide Daskor 440 (0.250 ng/μL) - ISD, pesticides in combination (0.125 ng/μL) – FGD+ISD and untreated control (CTL). For the control bees, syrup (water + sugar, 1:1) was offered without adding pesticide. Mortality was recorded daily until the last bee died, and statistical analysis was performed using the Kaplan-Meier Survival Analysis: Log-Rank method with the Holm-Sidak multiple comparison test. After 47 days for *A. mellifera carnica*, the results showed decreased longevity ( $<0.0001$ ) of bees exposed to pesticides being FGD (8 days), ISD (7 days), and FGD+ISD (10 days) when compared to CTL (17 days). Regarding *B. terrestris*, the exposure time was 26 days, and we also observed longevity decreasing ( $<0.05$ ), FGD (8 days), ISD (7 days), and FGD+ISD (7.5 days) in comparison to CTL (12.5 days). The outcomes suggest that the tested pesticides impacted the survival rate of both studied species. Considering the biological characteristics and the degree of sociability, *B. terrestris* may be more endangered since it can affect colony homeostasis, which is smaller. These data will be used for further new experiments with other approaches.

## 2.05.P-Tu070 Behavioral Evaluation of Imidacloprid's Repellency Capability in the Stingless Bee *Melipona quadrifasciata* (Apidae: Meliponini)

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Stingless bees such as *Melipona quadrifasciata* are exposed to pesticides found in fragmented habitats where they usually forage. Pesticides are recognized as an important driver of pollinator declines. Aiming to evaluate the immediate repellency capability of the stingless bee *M. quadrifasciata* to imidacloprid-treated food, bioassays were performed through a behavioral approach. Individuals of *M. quadrifasciata* were collected at the entrance from three non-parental healthy managed colonies (Sorocaba city, SP State, Southeastern of Brazil) and kept in laboratory conditions (28±2°C, 70±5% humidity) in cages. We analyzed behavioral parameters of locomotion (average speed, distance traveled, time in motion, rotation frequencies) and choice of food resource of *M. quadrifasciata* between feeders with and without the neonicotinoid insecticide imidacloprid in an arena. Arena contained two feeders (300 µL capacity) positioned on opposite sides with a distance of 10.5 cm between them. The feeders were filled with: food containing 0.01 ng/µL of imidacloprid (10 ppb), food containing 10 ng/µL of imidacloprid (10 ppm), pure food (only sucrose solution 50% m/v), and/or sucrose solution + acetone solvent. Each bee (15min fasting) was individually transferred to the arena (N = 12 bees per colony) and filmed for 10 minutes in 12 different scenarios of food choice. The videos analyzed by EthoVision software and the obtained data were submitted to generalized linear models (GLM) to analyze the influence of insecticide presence in the behavior responses of bees. The distributions used in the GLMs were gaussian, quasipoisson, and binomial negative. Analysis of data showed absence of repellency to imidacloprid. Food contaminated with both concentrations of imidacloprid showed no difference in the frequency of bee revisits compared to food without insecticide. Bees remained longer on food contaminated with 0.01 ng/µL of imidacloprid than on food without this insecticide (control). Imidacloprid interfered exclusively with the movement time of the exposed-bees inside the arena, which significantly was increased probably because of the initial superstimulation induced by the neonicotinoid after its immediate ingestion. These data demonstrated that stingless bees cannot control their exposure to neonicotinoids in food, thus the treating flowering crops with neonicotineoids could present a hazard to this native bee species.

## 2.05.P-Tu071 Comparison Between Two Neotropical Solitary Bees as Potential Model Species for Ecotoxicological Assays

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Toxicological data about the social honeybee are used in pesticide regulatory processes in Brazil, which does not cover the great diversity of Neotropical solitary native species. In order to indicate surrogate species of *Apis mellifera* for pesticide risk assessments, we compared two solitary bees, *Centris analis* and *Tetrapedia diversipes*, to verify their application for ecotoxicological bioassays in South America. Wooden trap-nests for both species were installed in southeastern Brazil. Each trap-nest contained 48 holes containing a paper straw in each one. Once a month, half of the closed nests by founding females were collected and transferred into laboratory conditions (28 ± 2°C; 65 ± 10% humidity). In both species, the nest contained an average of two to three brood cells built, and a sexual ratio with the number of males almost twice that of females. Individuals of *C. analis* emerged at a shorter time after the nest collection than *T. diversipes*. Furthermore, the distance of time between the first to the last individual of the same nest of *C. analis* (1.42 ± 0.24 days) is lower than *T. diversipes* (3.51 ± 1.05 days). According to this parameter, *C. analis* showed more advantages than *T. diversipes* because the use of individuals of the same age is recommended for ecotoxicological assays. Another disadvantage of *T. diversipes* is the higher parasitism ratio (13.65 ± 3.93%) and lower emergence ratio (54.5 ± 5.67%) by nest than *C. analis* (6.41% ± 2.66% and 78.4 ± 6.06 %, respectively). These factors may influence the total number of individuals obtained from the nest collection, which reached an average per year of 368.33 ± 87.35 bees of *C. analis* and 182.33 ± 59.4 of *T. diversipes*. Besides, the nest viability rate of *C. analis* was 89.88 ± 6.16% and of *T. diversipes* was 66.11 ± 14.5%. Although, under laboratory conditions, adults of *T. diversipes* showed easier maintenance than *C. analis*, with a higher survival rate and longevity. *T. diversipes* is a small bee and exhibits slower behavior than *C. analis*, a fast and of medium size bee, which had a higher food consumption. The higher consumption rate per bee may increase its exposure to pesticide residues. Concluding, both species showed advantage and/or disadvantage in laboratory conditions. Adjustments are necessary in order to choose the best model species for Neotropical solitary bees in ecotoxicological assays for pesticide risk assessment.

## 2.05.P-Tu072 Evaluation of Oral Toxicity of Abamectin Pesticide for Bees Native to *Scaptotrigona postica* Species and its Subeffects

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Bees are insects of great importance for biodiversity and agricultural production, since they are one of the responsible for promoting pollination, fruit generation and even providing honey, wax, propolis and pollen. In Brazil there is a wide variety of native bees known to have no stinger and poison, they are responsible for pollinating a wide variety of plants of the Brazilian ecosystem. Focusing on bees popularly called Mandaguari (*Scaptotrigona postica*), its role as pollinating agent is quite efficient,

since the amount of individuals per hive can reach more than 20 000 bees, thus positively impacting the pollination process of Brazilian forests. However, with the growing global demand for food, combined with economic interests to increase agricultural production, there is a growing use of pesticides, such as abamectin, which is considered highly toxic to bees and classified as dangerous to the environment. This substance is often used via foliar spraying in cotton, peanut, potato, among others. This fact can contribute to the decrease in the bee population and cause contamination of bee products. Considering that there are few studies related to the effects of pesticides on native bees, the present study aimed to determine the lethal concentration of abamectin (CL<sub>50</sub>; 0.0023 g L<sup>-1</sup>) as well as its lethal dose (DL<sub>50</sub>; 0.0093 µg a.i./bee) for the species *Scaptotrigona postica*, as well as the high rate of mortality and absence of flight ability of organisms against the high doses of the contaminant. Mandaguari's protein profile and the interference of the same contaminant in insect metabolism were also analyzed through the biochemical electrophoresis test in polyacrylamide gel, verifying the intensification of some enzymes such as catalase and peroxidase in individuals exposed to the contaminant, a strategy used by bees for detoxifying and tolerance.

#### **2.05.P-Tu073 Poisoning or Starvation? – Investigation of Deaths in *Apis mellifera* in a Chronic Laboratory Study**

**Timm Knautz**, Annika Barme and Tatsuya Sekine, *ibacon GmbH, Germany*

Repellent effects and food refusal are commonly known challenges in the conduction of oral biotests. The poor palatability of some pesticides leads to avoidance behaviour in bees. Reduced ingestion of the test item by test organisms not only causes difficulties in the calculation of effect doses due to nonlinearity in the dose-response relationship but also complicates the interpretation of data, whether mortality is caused by a direct test item effect or due to starvation.

To get more clarity on this topic the aim of this study was in a first experiment to define a threshold value which honeybees are suffering from starvation and are likely to die without influence of a toxicant. The first experiment was based on the chronic honeybee laboratory study (OECD Guideline 245) replacing different toxicant doses by different food quantities of 50 % w/v sucrose solution (5.0, 10.0, 15.0, and 20.0 mg feeding solution/bee/day). The amount of the daily administered feeding solution was adjusted to the number of living bees per test unit. In one additional treatment bees were starved for 6 hours (maximum oral application time for the acute honeybee test) and fed *ad libitum* afterwards. Mortality was assessed in a 24 hours interval. Absence of food for up to 6 hours had no adverse effect in this experiment, lethal starvation effects in acute honeybee studies can thus be excluded. A threshold of 10 mg feeding solution/bee/day was the minimum amount of food needed without long-term starvation effects on honeybees. In a second experiment a test item was administered to the bees with and without the option of an additional untreated feeding solution inside the test units (based on an OECD 245 study design). High mortality and reduced food uptake (< 10 mg feeding solution/bee/day) were observed in the upper concentrations when only test item treated feeding solution was administered to the bees. In contrast, mortality was significantly lower when an additional untreated feeding solution was present in the test unit. This indicates a strong repellent effect of the test item. Considering the previously determined starvation threshold the high mortality is likely to be a result of starvation or a combination of both factors rather than a pure test item effect. This result can facilitate the interpretation of chronic toxicity data and help to distinguish between toxicant related effects and starvation effects.

#### **2.05.P-Tu074 Defining Habitat Scenarios at Zonal Level for the Environmental Risk Assessment of Plant Protection Products to NTAs**

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The impact of PPPs on NTAs in agricultural landscapes depends on the particular landscape characteristics (i.e., composition and configuration) and complex spatial and temporal population dynamics, including 'action at a distance' or 'source sink' phenomenon. Some NTAs have high mobility and can disperse across the landscape, while others have low mobility and limited dispersal. Therefore, NTAs differ in their range distribution, with some taxa only being found in one type of habitat in the landscape whereas others may range across multiple habitat types including in-crop and off-crop. In addition to individual range differences, NTAs will be adapted to specific climatic ranges and habitat types, which combined with variations in phenology and life-histories, will result in spatial and temporal variation in the species composition of NTAs in agricultural landscapes.

We present the methodology adopted to develop habitat scenarios at zonal level for NTAs (pest control agents and wild pollinators) in targeted crops and present two exemplary cases for two crops in the northern (potatoes) and southern (olive groves) zones.

Habitat scenarios for each focal crop at each zone were derived based on high spatial resolution open source ALMaSS landscapes by applying a developed algorithm that randomly selects 1000 points of the focal crop, and extracts information on the habitat configuration surrounding each point. Two radius buffers were selected according to the typical activity range for pest control agents (predators and parasitoids) and wild pollinators. Based on the information retrieved, four types of habitat scenarios were defined: "simplified" (habitat with a high proportion of the focal crop); "agricultural dominated" (habitat with a high proportion of other crops); "natural habitat dominated" (habitat with high proportions of natural habitats, e.g., forest, hedgerows, beetle banks, etc); "diversified" (made up of similar proportions of the former categories). The habitat composition on each type of habitat scenario is represented by the 95th percentile composition based on all information collected.

On the two exemplary crop cases (potatoes and olive groves), the information on each habitat scenario was crossed with available information on NTA species assemblages common on those habitats, helping to define representative vulnerable NTA species to be used in risk assessment of the use of PPPs on those crops on those zones.

## **2.06 Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part I: Effects, Adaptations, *in vitro*, Population-Level)**

### **2.06.T-01 Effects of Climate Change and Emerging Contaminants on Caddisflies: Insights from a Multiple Stress Experiment**

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Freshwater biodiversity, ecosystem functions, and services are changing at an unprecedented rate due to the impacts of vast number of stressors overlapping in time and space (e.g., habitat alterations, pollution, climate change). Ecological impacts of emerging contaminants (ECs) such as pharmaceuticals (PhACs) and endocrine disruptors (EDCs) on freshwater ecosystems are not fully understood due to a large number of compounds present in the environment, as well as complexity of these ecosystems. Warming related to climate change can increase the toxicity of some chemicals in water making them more dangerous. Thus, such a combination of stressors may have ecological impacts on aquatic organisms like freshwater insects, which perform important ecological functions linking the freshwater and terrestrial ecosystems. Our study aimed at characterizing effects of individual and combined stressors such as pollution with PhACs and EDCs and increased water temperature on first level consumers (shredding aquatic insects) in freshwaters. We conducted the microcosm experiment with a simplified freshwater food web containing moss (Bryophyta) and a shredding caddisfly larvae of *Micropterna nycterobia* (Trichoptera). The experiment was conducted in the randomized factorial design, with four treatments: control (C), increased water temperature +4 °C (T2), ECs mix (EC), and multiple stressor treatment (MS = EC + T2). The ECs mix was composed of 15 PhACs and 5 EDCs belonging to different classes, and concentration of each compound was kept at 500 ng/L. Negative effect of increased water temperature on development of *M. nycterobia* was observed as decrease in body weight of larvae and earlier emergence of adults. The lipidome of *M. nycterobia* adults was also mainly affected by the temperature increase in both males and females. However, the presence of PhACs and EDCs in water had higher impact on metabolism of aquatic life stages of *M. nycterobia* than water temperature increase. Multiple stressor effect was recorded in *M. nycterobia* adults, both in metabolic response, and as a decrease in total lipid content. Thus, combined effects of increased water temperature and presence of PhACs and EDCs in water negatively impact population dynamics of aquatic insects, but they also indicate a reduction in biomass and resource quality for both, aquatic and terrestrial food webs.

### **2.06.T-02 Using a 30-Year Macroinvertebrate and Chemical Record to Discover What Drives Biodiversity in English Rivers**

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National macroinvertebrate diversity has steadily improved in England over the past 30 years, but why? In this study we matched macroinvertebrate records over 30 years with location, chemical, and physical records. The measurement locations and sample timings were different, so this required considerable effort to reconcile 62 000 biological records with about 500 000 chemical (and physical) records. We selected 1519 macroinvertebrates sites across England from the Freshwater river macroinvertebrate surveys (Biosys) dataset, on the basis of their long and consistent records. The kick sampling methodology used by the EA has not changed over 30 years. The analysis focused on family richness which is an indicator of the overall taxonomic community status and also Ephemeroptera, Plecoptera, Trichoptera (EPT) family richness which is the total number of family within three major orders of stream insects that are sensitive to water pollution. In this exercise, we matched macroinvertebrate sites with the nearest chemical measurement sites from the WIMS dataset. Of these, 28 variables offered data where >50% records were above an LOD. We used GLMM statistical analysis to identify which stressor or stressors can best explain the variability in macroinvertebrate family richness over 30 years in rivers with greater than, or less than, 5% wastewater content. All possible combinations were compared by GLMM which required up to 26 000 different model runs. It was found for both rural and urban rivers that zinc played the strongest role in controlling variance. What was extraordinary was that the zinc influence extended below EQS levels. All the models with all possible combinations of the chemical variables could explain deviance up to 40% for urban areas and up to 33% for rural EPT family richness and 27% for macroinvertebrate family richness (worms, mollusks, insect larvae etc.) in the higher wastewater (urban) sites and up to 21% in the low wastewater (rural) sites. Thus, these variables do not exclusively explain the variance. If another chemical played a role, that was not measured, it must be one that has declined in concentration. This study represents an attempt to 'listen to nature'. Rather than presume or infer what factors or chemicals suppress biodiversity, we have used a vast record of biological and chemical monitoring to tell us what matters to wildlife. Potentially this approach turns the way we regulate water quality to improve biodiversity on its head.

### **2.06.T-03 Pesticide Adaptation Increases Synergism Between Multiple Stressors in *Gammarus pulex***

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Aquatic organisms are confronted with multistress conditions that often cause nonadditive effects. Although scientific evidence for synergistic effects is mounting, we lack information on how pesticide adaptation or ecological memory shapes the multiple stress–response relationships. Here we collected *Gammarus pulex* from small streams with a wide range of pesticide contamination and exposed them to a mixture of neonicotinoid insecticide clothianidin and an azole fungicide prochloraz under different temperature regimes. Under optimal temperature (16°C), *G. pulex* with prior pesticide exposure in the field showed up to 3-fold higher tolerance to clothianidin alone and in mixture with prochloraz, as compared to the non-contaminated reference populations. The difference between pesticide tolerance of both groups decreased with increasing temperature stress. Although there was no synergistic interaction between clothianidin and prochloraz, effect was significantly stronger in reference populations. Further, the combination of both toxicants and temperature stress caused synergism shown by a model deviation ratio (MDR) = 4.0 even at 1 µg/L of prochloraz that is frequently detected in the environment. Under multiple stress conditions, interaction was stronger and more frequent in pesticide-tolerant populations. These synergistic interactions were quite well predicted by the stress addition model (SAM). Our findings demonstrate that heat stress interacts with mixture toxicity and causes even stronger effects on pesticide adapted organisms. Therefore, the impacts of pesticide cocktails on the ecosystems under global warming cannot be overlooked.

**2.06.T-04 Springtail Mortality in Response to Sequential Exposure to Chemical and Climate Change-Related Stressors**  
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In their environment, organisms are exposed to a combination of anthropogenic and naturally occurring environmental stressors. Temperature changes may affect the tolerance to toxicants of several terrestrial arthropod members of the soil community, making climate change relevant in understanding toxicity. Besides imposing physiological effects due to variations in temperature, climate change may also affect soil hydrology in a complex way, with effect varying regionally, from predicted increased precipitation in some regions to decreases in other. Thus, both waterlogging and drought may occur and affect soil community composition and performance. Species living on or near the soil surface are generally more drought tolerant than species inhabiting lower layers, and local conditions may further influence their tolerance. To understand how toxicant exposure followed by drought and temperature increase affects the soil invertebrate community, varying between climatic regions, species, and populations, we compared two springtail species from Arctic and temperate regions with contrasting drought tolerance, *Folsomia quadrioculata* being more sensitive to drought than *Hypogastrura viatica*. Two Arctic and two temperate populations from each species were first exposed to four sublethal concentrations of the neonicotinoid imidacloprid (0 – 0.1 mg/kg) in soil mesocosms at 15°C for 14 days, followed by different levels of drought stress (85 – 100 % relative humidity) at 15°C and 20 °C for 3 h (*F. quadrioculata*) or 7 days (*H. viatica*), assessing survival. Drought tolerance decreased with increasing imidacloprid concentrations in all populations from both species, and this effect was stronger in the temperate compared to the Arctic populations. Drought stress at a higher temperature further decreased survival in the most drought tolerant springtail species *H. viatica*, while this effect was not as strong in the less drought tolerant species *F. quadrioculata*. In conclusion, macroclimatic adaptations affected imidacloprid toxicity in Arctic vs. temperate springtail populations, but with increasing drought and temperature stress, these adaptations were not reflected in the mortality across populations and species. Thus, local adaptations to microclimate should be taken into account to better understand the responses to chemical stressors with concurrent exposure to other stressors.

**2.06.T-05 Synergic Effect of Nitrate Exposure and Heatwaves on the Growth and Metabolic Activity of Microalgae**  
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Aquatic ecosystems are threatened by climate warming as well as other anthropogenic stressors such as eutrophication by, e.g., nitrate. However, it remains unclear how nitrate exposure can alter the resilience of microalgae to climate warming in general, and heat waves in particular. Any change in community structure or abundance of microalgae can affect the overall energy flow of a freshwater ecosystem. To get a better understanding of these processes, the effect of elevated temperature and nitrate pollution on growth, metabolites (sugar, and protein), oxidative damage (lipid peroxidation), and antioxidant (polyphenols, proline) accumulation within the cells of *Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata* were investigated. A 3 × 3 factorial design was used, where microalgae were exposed to one of three ecologically relevant levels of nitrate (5, 50, or 200 mgL<sup>-1</sup> NO<sub>3</sub><sup>-</sup>) at temperatures of 20 °C for 2 weeks. Subsequently, two heatwave scenarios were imposed: a short heatwave at 24°C for 2 weeks, and a long heatwave with an additional 2 weeks at 26°C. A positive synergic effect of heatwaves and nitrate on the growth and metabolic activity was observed in both algae. In the short heatwave scenario, this oxidative damage was controlled by increases in antioxidant (polyphenols, proline) levels but in the continuing long heatwave, the antioxidant (proline) response decreased. The high growth rates, high metabolic activity, low oxidative stress, and high antioxidants during short heatwaves in moderate nitrate (50 mgL<sup>-1</sup>) seem to increase the food availability to zooplanktonic grazers in a sustainable way. On the other hand, long heatwaves in high nitrate conditions seem unsustainable due to the increased oxidative stress and relatively low antioxidants (proline), increasing the risk for massive algal die-offs. Heatwaves and nitrate can also affect the community structure of the microalgae as both microalgae show similar changes but to a different magnitude.

**2.06.P Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part I: Effects, Adaptations, *in vitro*, Population-Level)**

## 2.06.P-Mo108 Impact of Warming on the Sensitiveness of *Artemia franciscana* to the Pesticides Oxyfluorfen and Copper

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Nowadays, environmental concerns are not only a worry of the scientific community but also an increasing worry of the politician and social communities. Climatic changes assume a terrific position in politician programme, since the effects undervalue until now are showing to be real and it is increasing the frequency and severity of extreme events as floods, wildfires, storms, hurricanes, likewise the temperature increase, well significant in some world regions. The increase of the human population is also a main concern, and it is critical search for solutions to produce enough food to feed everyone. Pesticides have been used as an easy solution to achieve large amounts of food, since they may prevent pests and consequently increase the natural production. However, their overuse has been reported as dangerous to the ecosystems and its communities, with noxious impacts not only on the soil but also in aquatic surrounding systems. By this, this work proposes to: a) determine the lethal concentration of oxyfluorfen and copper to the zooplankton *Artemia franciscana*, at different temperatures (15°C, 20°C and 25°C); b) understand the biochemical impacts, namely on the sugars composition, of the tested chemicals at the different temperatures scenarios, on *A. franciscana*; and c) evaluate the impact of the climatic changes, particularly the temperature increase, on the brine shrimp sensitiveness to the tested pesticides. To achieve the aims, the organisms were exposed to a concentration range of oxyfluorfen and copper, individually, at the three temperatures per 48 hours to determine the lethal concentration of each chemical at the different temperatures. Moreover, sublethal bioassays were conducted for 7 days to understand the effects of the compounds at the different temperatures on the biochemical profiles of *A. franciscana*. Acute tests showed that the chemicals toxicity increase with the temperature increase, and oxyfluorfen reveals to be more noxious to *A. franciscana* than copper. Considering the biochemical impacts, glucose and mannose were the dominant sugars in *A. franciscana* profiles, regardless of the temperature and treatment. Furthermore, significant differences were observed among temperatures, being the highest differences between the organisms exposed to 15°C and the ones exposed to 25°C. This study highlights the dangerous impacts of warming at lethal level, but also at the nutritive value, exhibiting significant changes on sugars profiles and thus on energetic sources.

## 2.06.P-Mo109 Physiological Effects of Microplastic During Simulated Marine Heatwaves in the Mediterranean Coral *Astroides calycularis*

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Climate change and pollution are impacting marine habitats, both directly and indirectly. Among them marine heatwaves and microplastic are recognized as global threats, especially in the Mediterranean basin where environmental changes are happening at faster rates. In this study, we studied the effects microplastics during simulated heatwaves on respiration and excretion rates were studied in the Mediterranean coral *Astroides calycularis*. Coral colonies were exposed to four different conditions (presence/absence of microplastics and ambient/increased temperature) for four weeks under laboratory conditions, increasing the temperature by 1 °C/week (from 26 °C to 29 °C), and maintaining the 29 °C peak for 1 week. During the exposure, oxygen consumption was measured for each colony using respiration chambers. An aliquot of seawater was collected from each chamber for the ammonia excretion analysis, and mortality rate was also measured. ANCOVA (polyps/colony as covariate) was applied to highlight significant interactions between microplastics and heatwaves on physiological rates during a 4 weeks' exposure, while ANOVA was used for testing differences in the mortality rate. Mortality was not significantly different among conditions and physiological rates increased with temperature. Respiration and excretion rates showed a bell-shape trend, with higher values after 3 weeks, at 29 °C, probably due to the proximity to the warm sublethal thresholds, being around 24-29 °C according to the literature. The decrease detected for both rates after four weeks could be due to acclimatisation to laboratory conditions or to adaptation: if the latter case, reducing metabolic rates is a strategy for energy conservation to better survive in a stressful scenario. Increased temperature was the main factor affecting metabolic rates, while the presence of microplastics only reduced respiration rate after 3 weeks of exposure. Our data suggest that temperature between 27 °C and 29 °C is probably within a zone of metabolic thermal compensation for the species.

## 2.06.P-Mo110 Impacts of the Cumulative Effect of Copper Exposure and Ocean Acidification on Cold-Water Octocoral *Viminella flagellum* – A Transcriptional Approach

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Deep-sea mining is one of the potentially most threatening activities for the marine environment. The mechanical and toxic effects originated by the sediment plumes is described as highly deleterious for the deep-sea organisms. In parallel, the excessive amounts of CO<sub>2</sub> that have been absorbed by the oceans is leading to a decrease in the pH of seawater, named ocean acidification (OA). This phenomenon is expected to severely affect seawater chemistry (e.g., trace metal speciation) restricting calcification of marine organisms and favouring erosion of habitats created by marine calcifiers, such as Cold-Water Coral (CWC) species. The characteristics of long living and slow growing make CWC particularly vulnerable to anthropogenic impacts. The octocoral



*Viminella flagellum* is one of the most important structuring species in the Azores. The polymetallic sulfide (PMS) particles originated from mining activities plumes have adverse consequences for *V. flagellum* communities, such as the polyps smothering and clogging and the toxic effect of copper (Cu) that is one of the most released metals from the PMS particles. How can vulnerable CWC overcome the cumulative effect of Cu exposure, under a mining activities scenario, and OA? To address this question, four OA/Cu-contamination scenarios were used: (1) ambient pCO<sub>2</sub>/pH level as measured in the in-situ conditions (385 µatm/pH 8.09); (2) high pCO<sub>2</sub>/reduced pH (1000 µatm/7.73); (3) ambient pCO<sub>2</sub>/pH level and additional Cu concentration (60 µg/L); (4) high pCO<sub>2</sub>/reduced pH and additional Cu concentration (60 µg/L). The pH/pCO<sub>2</sub> modification was achieved by bubbling seawater with either pure CO<sub>2</sub> (to increase pCO<sub>2</sub>) or CO<sub>2</sub>-free air (to decrease pCO<sub>2</sub>). The results show a Differential Gene Expression (DGE) between treatments. Our experimental results indicate that it is Cu exposure that modulates *V. flagellum* gene expression rather than seawater acidic conditions. Also, the cumulative effect of both seems to suppress most of the genes expressed under noncumulative treatments. In this work we discuss the molecular effects of the interactive effects of anthropogenic activities on CWC and the importance of identifying genetic biomarkers of physiological stress for future studies of environmental risk assessment.

## **2.06.P-Mo111 The Influence of Salinity on the Toxicity of Chemical UV-Filter 4-MBC to Sperms and Adults of the Free-Spawning Mussel *Mytilus galloprovincialis* (Lamark, 1819)**

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In marine-coastal systems, organisms are exposed to a growing number of stressors, with associated ecotoxicological risks at different life stages and ecological levels. Considering the nature and different mechanisms of actions of stressors, their co-occurrence can result in possible interactive effects. Among stressors, changes in environmental abiotic factors related to Climate Change (i.e., salinity shifts) or new pollutants (i.e., personal care and cosmetic ingredients) have been identified as key emerging concerns. Particularly, 4-methylbenzylidenecamphor (4-MBC), as one of the most used UV-filters in several products, tends to be continuously discharged into aquatic environments, posing a high risk for inhabiting organisms. However, the toxicity exerted in nontarget species at different biological levels is almost unknown, especially under predicted Climate Change (CC) scenarios. Thus, the present study aimed to clarify if and how salinity variations (S 20, 30, and 40) potentially modulate impacts of 4-MBC environmentally relevant and slightly high contaminations (1, 10, and 100 µg/L) on different biological levels (male gametes and adults) of *Mytilus galloprovincialis*. By adopting *in vivo* and *in vitro* approaches, physiological and biochemical endpoints were assessed in short- and long-term assays, providing ecologically more relevant information on organism responses. Results showed that salinity acting alone posed the greatest impairments on both biological levels at the lowest (20) and highest (40) tested levels. When salinity acts as a co-varying stressor (salinity-dominant interaction), enhanced effects resulted evident, inducing a greater redox status imbalance and oxidative stress in both stages. The major toxic UV-filter impacts were detected in the worst salinity conditions (20 and 40), with main effects in terms of: I) sperm structural impairments, motility and kinetic alterations, DNA damage, and shortened longevity; II) physiological dysfunctions, metabolic capacity and energy reserves impairments, oxidative DNA damage as well as oxidative and biotransformation enzyme activity variations at adult stage. Additionally, a salinity-dependent uptake of waterborne 4-MBC was detected after 28 days exposure. Overall, it stands out that salinity influences biological pathways and, thereby, reinforces the high potential ecological risk of UV-filter environmental contaminations on *M. galloprovincialis*, especially in an expected salinity stress scenario.

## **2.06.P-Mo112 Integrative Approaches Highlight the Adaptive Potential and Plasticity of the Invasive *Hemigrapsus sanguineus* and *H. takanoi* While Outperforming *Carcinus maenas* in Europe**

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Ecosystem services are increasingly relevant in policymaking and threatened due to overexploitation, climate change, pollution, and invasive species. Besides human-mediated introduction, the success of coastal bioinvasions is influenced by ecosystem disturbances. The plastic capacity of invaders then became an increasing matter of concern under such rapidly changing environments.

Predatory crabs are among the most conspicuous marine invaders: Asian shore crabs (*Hemigrapsus takanoi* and *H. sanguineus*), native along northwestern Pacific rocky shores, established viable and dense populations throughout northern European waters. Detrimental effects were reported on native crab species (*Carcinus maenas*), food webs, and bivalve spat - posing a threat to natural stocks and aquaculture. *Hemigrapsus* spp remain unreported in southern Europe, where suitable habitats reach higher temperatures: their ecophysiology is fairly unknown, namely under warming or pollution scenarios.

This project investigated the adaptative potential and plasticity of native and invasive crabs by addressing exposure responses to 1) warming (chronic), 2) a new generation pesticide (acute and chronic), and 3) a heatwave (acute thermal stress) and pesticide LOEC (bifactorial) at the organism level – bioassays concerning lethality, behaviour, growth, feed intake, or respiration – and

sub-individual level – enzymatic and biochemical assays related with detoxification, oxidative stress and related damage, energy metabolism, and neurotransmission.

The results pinpoint that warmer regimes may not deter *Hemigrapsus* spread to Southern European coastal areas. In addition, the native *C. maenas* was more impacted by the exposure to a new generation pesticide. These findings suggest that, in the *C. maenas* native range, *Hemigrapsus* seem to have higher plasticity under global change scenarios, prompting the native displacement, and expected dominance in the invaded range.

Addressing the impacts of global change drivers is relevant to unravel the underlying mechanisms for bioinvasions success and facilitate informed management measures in some of the most worrying topics to environmental sciences and society.

#### **2.06.P-Mo113 Light Pollution Increases Sensitivity of an Aquatic Insect to Thermal Stress and Pesticide Exposure**

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Urbanization is steadily increasing worldwide imposing novel challenges on urban/suburban aquatic ecosystems. As an example, light pollution has become an emergent issue of environmental concern since many health and ecological detrimental effects artificial light at night (ALAN) have been identified.

Besides light pollution, urban aquatic ecosystems are usually recipients of runoff from municipal and industrial discharges resulting in increased concentrations of chemical contaminants (including pesticides). Moreover, considerably warmer temperatures are observed in urbanized aquatic ecosystems (“urban heat islands”) due to urban air and ground temperatures, paved surfaces, and decreased riparian vegetation. Although ALAN, warming, and chemical contamination are common stressors present in urban areas, their interaction and combined effects are not studied. Here we present preliminary data testing for the hypothesis that exposure to low and environmentally relevant levels of ALAN can change the upper thermal tolerance and sensitivity to pesticide exposure of *Chironomus riparius* larvae.

To assess thermal tolerance, we determined the critical thermal maximum (CT<sub>Max</sub>) in *C. riparius* larvae after exposure to three ALAN conditions (0, 1, and 10 lux during nighttime). The results show that ALAN decreases the upper thermal tolerance of *C. riparius* larvae (CT<sub>Max</sub>). Pesticide tolerance was evaluated through survival curves (time-to-event analyses) where *C. riparius* larvae reared in the same ALAN treatments (0 and 10 lux during nighttime) were exposed to a lethal Glyphosate (Roundup) concentration. Mortality was evaluated every 30 min over 8 hours. Significantly different survival curves indicate a higher sensitivity of *C. riparius* larvae previously exposed to ALAN. Ongoing experimental work is investigating the physiological basis of decreased thermal tolerance (heat shock proteins and oxidative stress/ damage) and also validating results concerning increased sensitivity to pesticides with other compounds (neonicotinoids, pyrethroids). Our results suggest that for a better assessment of ecological effects of climate change and of chemical contamination in urban aquatic ecosystems, it is crucial to consider anthropogenic-induced changes to light conditions.

#### **2.06.P-Mo114 Using a Dynamic Energy Budget (DEB) Model to Analyze the Sublethal and Lethal Effects of Insecticides at Different Temperatures**

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Temperature is an important factor for biological processes, i.e., by affecting growth, feeding, and reproduction, especially for aquatic ectothermic species. Furthermore, temperature changes the behaviour of chemicals, such as their fate and bioavailability in the environment. The importance of gaining a better understanding of how temperature affects chemical toxicity is emphasized by the observation that many previous studies have found that the acute lethal toxicity of chemicals increases with temperature. Although a better understanding of the impacts of temperature on lethal and sublethal endpoints is important, few studies have been conducted, and the underlying mechanisms of temperature effects are largely unknown.

Temperature can affect the toxicity of chemical substances by effects on the kinetics: temperature affects the uptake of chemicals, and therefore (apparent) toxicity could be changed. Also, the intrinsic sensitivity of organisms can be influenced by altering certain gene expressions or enzyme activities. To understand these processes and allow to separate the drivers, toxicokinetic-toxicodynamic (TKTD) models like the Dynamic Energy Budgets (DEB) models are needed for lethal and especially sub-lethal endpoints.

In this study, we have applied DEB models to data on the lethal and sublethal effects of imidacloprid and flupyradifurone on *Gammarus pulex* at different temperatures. Our results showed that for both insecticides, the dominant rate constant and threshold value tend to decrease with increasing temperature for both lethal and sublethal effects, while the effect strength and background mortality tend to increase with increasing temperature. The results showed that temperature influences both the kinetic of chemicals and the intrinsic sensitivity of organisms. In addition, a 180-day experiment is currently being performed to capture the effect of temperature on mortality and growth. The preliminary results suggest that the DEB model fits well and that damage from imidacloprid is diluted by growth.

Overall, the current results indicate that the relationship between each parameter and temperature might vary with species and chemicals; therefore, more studies need to be conducted to get a general and clearer picture. Also, this present study deepens the understanding of the mechanism of temperature on pesticide toxicity, which can be used for future risk assessment when the temperature is integrated.

## **2.06.P-Mo115 DOM Quality and Origin Does Not Greatly Affect Uptake and Accumulation of Lipid Soluble Contaminants in Two Coastal Filter Feeders**

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Coastal darkening, the increased input of darker terrestrial derived dissolved organic matter in coastal system, has been a growing environmental concern in recent years.

Terrestrial dissolved organic matter (terrDOM) is overall comprised of larger humic molecules with stronger amphipathic properties compared to marine DOM (marDOM) and thus has a more favourable adsorption profile for lipophilic contaminants. Previous studies show that the presence of terrDOM leads to reduced concentration of contaminants in the open water column and less uptake directly from the water (bioconcentration), thus indicating that some contaminants adsorb well to terrDOM. However, the very same properties may lead to an increased contaminant uptake via feeding processes, especially in filter feeding organisms. Thus, we expected higher bioaccumulation rates in filter feeders exposed to terrDOM than marDOM. To test our hypothesis with two mechanistically different filtration types (cirri-trapping vs. mucus feeding), we exposed blue mussels (*Mytilus edulis*) and subsequently ascidians (*Ciona intestinalis*) to teflubenzuron - a lipophilic (log K<sub>OW</sub>: 5.39) veterinary drug commonly used in aquaculture against sea lice infestations - under four different DOM conditions. We employed a factorial design that was comprised of 24 aquaria in each of the two experiments. The different DOM types used include DOM derived from phytoplankton (marDOM), DOM derived from forest leaves (terrDOM), a mix of the two (mixDOM), and seawater with low natural background DOM. All seawater was filtered to 0.2 µm before potential DOM addition.

Contrary to expectation, our results showed that terrDOM exposure did not lead to the highest bioaccumulation in any of the two taxa, but rather a trend toward higher bioaccumulation with marDOM or mixDOM exposure. However, terrDOM exposure also did not lead to lower bioaccumulation than the seawater only treatment either. Bioaccumulation of teflubenzuron was overall higher in blue mussels than ascidians.

## **2.06.P-Mo116 Analytical and Risk Assessment Monitoring of Drinking Water Treatment Plants in Biscay (Basque Country)**

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The quality of drinking water shows increasing safety requirements and the highest standards to ensure human health. In the EU the legal framework is governed by the Drinking Water Directive (DRW, 2020/2184), including the first watch list (2022). However, we can only find a list of parametric values for less than 20 organic micropollutants. Therefore, there is a clear need for more comprehensive knowledge about the presence of emerging pollutants and the potential toxicological hazard to human health in raw and treated waters.

In this scenario, the Water Council of Bilbao-Bizkaia (CABB), together with researchers of the UPV/EHU, Gaiker and Cadagua, led the project *EmerGen* to screen some of the most sensitive drinking water treatment plants (DWTPs) in Bizkaia (Basque Country, Spain). For that, we monitored the quality of 5 raw and treated waters over 2 years, including an experimental pilot plant designed to reuse the water from the Nervión-Ibaizabal River in Bilbao in the event of a serious lack of the typical supplies in summer. The aim of this work was to monitor sensitive and demanding DWTPs in Bizkaia to assess the quality of the raw and treated waters in terms of the presence of contaminants of emerging concern, and the toxicological effects such as mutagenicity. In addition to this, the efficiency of the implemented water treatments was also considered, especially when the eventual supplies cannot ensure the expected requirements.

Ten monitoring campaigns were carried out from July 2020 till May 2022, one per season and two in summer. An automatic large-volume solid-phase extraction system (LV-SPE, MAXX Mess-u Germany) holding a triphasic cartridge (WAX, WCX, HR-X) was used to sample 20-40 L from each DWTP. Water extracts were injected in a Thermo Scientific Dionex UltiMate 3000 coupled to a UHPLC-q-Orbitrap (Focus) (Thermo Scientific) operating in full scan-data dependent MS2 acquisition mode. Two independent data handling strategies were implemented: multitarget screening of CECs and nontarget screening. With these quantitative data, we estimated the risk quotient (RQ) values based on PNEC values available in the NORMAN database

The experimental treatment plant in Etxebarri exhibited the highest concentrations due to the contamination load of the river, and the RQ values for Telmisartan and Cortisone were >1, considered of concern. In the other 4 DWTPs, the ranges of the concentrations are within <0.1 -30 ng/l and the RQs were significantly lower.

**2.06.P-Mo117 Water Stress from Future Climate Changes and Modeling Impacts to the Environment from Consumer Product Chemical Exposure in the United States and Europe** Raghu Vamshi<sup>1</sup>, Brenna Kent<sup>1</sup>, Scott Dyer<sup>1</sup> and Andrea Carrao<sup>2</sup>, (1)Waterborne Environmental, Inc., (2)Kao USA

Growing human population in combination with expansive evidence of climate change has added stress on freshwater availability at a global scale. Water stress is impacting communities to focus on water conservation, reuse, and recycling technologies for freshwater use. However, they may require innovations in consumer products that depend on water for their function and disposal. Water stress across the two regions, United States (US) and Europe (EU), were examined by evaluating datasets considering historic, current, and future water availability and use scenarios. Inclusion of anticipated climate change events required datasets that incorporated scenarios of estimated future population and water use with high spatial resolution. These datasets captured predicted temporal trends for the years 2020-2050 and were integrated with wastewater treatment plant infrastructure data across the US using EPA's Clean Water Needs Survey and Europe using EEA's Waterbase. The consolidated information was used to develop current and future water use scenarios. The influence of future water use scenarios on down-the-drain chemical exposures were predicted by evaluating four consumer product chemicals with various phys/chem properties and use patterns. Results from the modeling provided a quantitative forecast of the potential impacts of water stress on down-the-drain chemical exposure and potential risk. Incorporating spatial and temporal variation in water stress and understanding its impact on the environment from chemical risk, through the lens of future scenarios, provides a new dimension in the development of consumer products. Incorporating these issues into product development, now, will ensure that both consumers and the environment will be appropriately stewarded, especially considering future environmental challenges in the US and EU.

**2.06.V Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part I: Effects, Adaptations, *in vitro*, Population-Level)**

**2.06.V-01 Global Climate Change Increases the Impact of Pollutant Mixtures in the Model Species *Paracentrotus Lividus*** Juan Ignacio Bertucci<sup>1</sup>, Carmen Veloso-Cerredelo<sup>1</sup> and Juan Bellas<sup>2</sup>, (1)Contaminación Marina, Centro Oceanográfico de Vigo - IEO- CSIC, Spain, (2)Centro Oceanográfico de Vigo - IEO- CSIC, Spain

The ingestion of microplastics (MP) at environmentally relevant concentrations has been shown to cause stress, false feeling of satiation, reproductive complications, and reduced growth rate in several aquatic organisms. Due to their inherent hydrophobicity, organophosphorus pesticides such as chlorpyrifos (CPF) are strong candidates to be adsorbed by MP in the environment. Oceans are also impacted by several aspects of global climate change such as the increase in seawater temperature -ocean warming (OW)- and their acidification -ocean acidification (OA)-. The goal of the present work is to study whether global climate change factors could increase the toxicity of pollutants on *P. lividus*. To achieve this, a fertilization assay was carried out following the United States Environmental Protection Agency guidelines, and a sea urchin embryo test (SET) was carried out to determine growth and alteration level of larvae, and the index of contaminant impact. For both assays, treatments consisted of a control with Filtered Sea Water (FSW, "Control"), MP 3000 particles / mL ("MP"), acidified FSW ("OA"), CPF at 250 µg/L, a combination of both MP (3000 particles / mL) and CPF (250 µg/L) (MP+CPF), and a treatment of MP+CPF under OA conditions (OA+MP+CPF). Each treatment was tested at two temperatures: 20°C and 24°C (OW conditions). The fertilization assay showed that the negative effect of CPF on the fertilization rate is higher when MP are present in the media, which may act as carriers. Also, the global climate change conditions predicted by FAO increase the sensitivity of embryos to MP and CPF. The highest growth inhibition of *P. lividus* larvae was observed in the treatment MP+CPF. At morphological level, our study shows that an increase in temperature increased the ratio POL/BL, which means that under the predicted OW conditions, the arms of larvae would be proportionally longer in relation to the rest of the body. The presence of CPF reduced the relative length of the arms, while the relative width of larvae was increased, that is, larvae exposed to CPF tend to adopt a rounded shape which is obviously detrimental to their normal buoyancy and could affect their ability to feed in the water column. The combination of CPF with other stressors seems to aggravate this situation. Our findings support the idea that global change conditions could have a severe impact on marine life, increasing the negative effect of toxic agents commonly present in the sea.

**2.07 Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part II: Microcosms, Mesocosms, Factorial Design, Community-Level)**

**2.07.T-01 Leaf Species-Dependent Fungicide Effects on the Structure and Function of Leaf-Associated Microbial Communities**

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Microbially mediated leaf litter decomposition is a vital ecosystem function in streams within forested areas, which can be affected by fungicides. However, fungicide effects on leaf litter decomposition have been investigated almost entirely with black alder leaves, a leaf species with traits beneficial to consumers (i.e., low recalcitrance and high nutrient content). At the same time, little is known about fungicide effects on microbial colonisation and decomposition of other leaf species with less beneficial traits. In this 21-day lasting study, we explore the effects of increasing fungicide sum concentrations (0 to 3000 µg/L) on microbial colonisation and decomposition of three leaf species (black alder, Norway maple, and European beech) varying in terms of recalcitrance and nutrient content. The rate of leaf litter decomposition, leaf-associated fungal biomass, and bacterial density were

quantified to evaluate potential effects at the functional and structural levels. In absence of fungicides, beech, as the species with the least beneficial leaf traits, showed a 50% lower decomposition rate than alder and maple. On the contrary, fungal biomass did not follow this pattern of leaf litter decomposition observed for the three leaf species, suggesting a decoupling of biomass and functional trait composition in fungal communities. In the presence of high fungicide concentrations (300-3000 µg/L), beech showed a concentration-related decrease not only for microbial leaf litter decomposition but also for fungal biomass. This suggests that beneficial traits of leaf litter (as for alder and maple) enable leaf-associated microorganisms to acquire leaf-bound energy more easily to endure potential effects induced by fungicide exposure. Our results point to the need to deepen our understanding on how leaf species' traits relate with the impact of chemical stressors on the leaf decomposition activity of microbial communities. This step seems relevant for a more complete understanding of anthropogenic effects on carbon and nutrient cycling in streams.

#### **2.07.T-02 Chemical Stress Increases Methane Production in Freshwater Sediments: Role of Temperature Feedbacks, Adaptation, and Resistance**

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The increasing emissions of atmospheric greenhouse gases are the main driver of climate change and a serious concern for human health and the environment. Microbial methane (CH<sub>4</sub>) production in anaerobic freshwater sediments is the largest and most unresolved source of atmospheric CH<sub>4</sub>. Given the rising CH<sub>4</sub> emissions, researchers have been investigating environmental controls and climate change feedbacks for decades. Despite their omnipresence, the impact of anthropogenic chemical stressors (e.g., antibiotics) on methanogenesis in freshwater systems is still largely unknown. Against this background, we first incubated natural pond sediment at four levels of a five-component antibiotic mixture. Second, three temperatures (i.e., 10, 15, and 20 °C) were employed as additional factor to assess interactions on the toxicokinetics and -dynamics of antibiotics. Third, the adaptability of a pristine and a preexposed microbial community was investigated. Both communities were treated with antibiotics for three weeks and consecutively incubated at three concentrations of the same antibiotic mixture. In every experiment, the effect of antibiotics was decisive with the CH<sub>4</sub> production rates almost doubling at the highest treatment concentration (i.e., 5000 µg L<sup>-1</sup>). Furthermore, higher temperatures resulted in increasing effects of antibiotics indicating a potential feedback-loop. Both the pristine and the exposed community showed effects of antibiotics on methanogenesis with effects increasing in the former and decreasing in the latter after the first treatment phase. Compound-specific isotope signatures indicated that the same synthesis pathways (i.e., mainly acetate and H<sub>2</sub>/CO<sub>2</sub> as substrates) were utilized and metabarcoding of the 16S rRNA gene suggests changes in the community composition of microorganisms relevant to methanogenesis.

#### **2.07.T-03 Seasonal Fluctuation of Metabolomic and Photosynthetic Yield Response of *in situ* Freshwater Biofilms Exposed to a Model Herbicide**

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With increasing aquatic chemical pollution, the study of microbial communities (e.i periphytic biofilms) improves the ecological dimension of biomonitoring. Despite a growing knowledge, there is a paucity of information about the seasonal fluctuation of their sensitivity to chemical stress. If classical endpoints often lack sensitivity and focus only on one component of the biofilm, untargeted metabolomics can provide a comprehensive and sensitive picture of the molecular response prior physiological. The present study aims to characterize the changes of sensitivity of freshwater periphyton to the model herbicide terbuthylazine over months through the combined measurement of the photosynthetic yield (ΦPSII) and the metabolomics response based on high-resolution mass spectrometry. To do so, periphytic biofilms were sampled on a pilot site and exposed during 4 h in controlled conditions to a range of six concentrations of herbicide. The sensitivity of periphyton to chemical was assessed through the determination of Benchmark Doses with a standard deviation of 1% compared to the control (BMD) and their cumulative distribution for metabolomics data. The results indicate a change in the sensitivity over the months for both endpoints. Indeed, BMD of ΦPSII vary from 5.5 to 13.8 µg/L. Multivariate analyses on metabolomics data showed a response at 0.3 µg/L of terbuthylazine; our results highlighted that the metabolomics response is more sensitive than the photosynthesis since almost 50% of the metabolome have reacted at the BMD ΦPSII. Metabolite identification found significant pathway modification under exposure. This study shows that sensitivity of periphyton to chemical stress fluctuates along the year, highlighting the need to consider it for field studies. In addition, this work confirms the higher sensitivity of metabolomics against photosynthetic response in the form of their BMD responses. The metabolite annotation highlighted similar pathway response effect but different regulation. The continuation of these investigations along the year will provide additional insight on the influence of environmental parameters on the sensitivity of periphyton to chemical stress. Especially, metabarcoding analyses should highlight the natural taxonomic shift according to environmental conditions and hone the identification of which metabolites and pathways are sensitive to environmental conditions against those specifically impaired by the chemical stress.

#### **2.07.T-04 Contamination on Ecosystems and Habitat Selection – The Role of Ecological Interactions on the Behavioural Responses**

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The reason by which ecotoxicity tests are reductionist regarding to ecosystems complexity is due to the fact that the environmental variables can be more easily controlled by the experimenter. In this sense, two important scenarios have not been usually integrated to ecotoxicology: i) the chemicals are not always homogeneously distributed in the ecosystems and ii) the effect of the ecological interactions (such as predation, competition, etc.) can exert on how organisms respond to toxicity. The aim of the present study was to assess how contamination can affect the spatial distribution of organisms in a chemically heterogeneous landscape and how the habitat selection in a contaminated environment is influenced by the interactions that organisms have with other ecological factors. To that end, three study cases were analyzed where contamination was evaluated along to stressors/attractors simultaneously in two nonforced free-choice multicompartimented assay systems: the linear and the Heterogeneous Multi-Habitat Assay System (HeMHAS). For the tilapia fry case, the organism avoided the contaminant, but when confronted to food the fish intermittently moved to the most contaminated compartment. For the freshwater shrimp case, the organisms avoided the contamination but this response was density-dependent inversely-proportional, so the higher density the lower avoidance, and conversely. For the estuarine shrimp case, it avoided the contaminant as well, but when confronted to predation risk, the response shifted. In addition, when organisms were faced to the attraction of food along to both stressors, the foraging behavior was impaired in the presence of contamination. As conclusion, the nonforced exposure systems (e.g., HeMHAS) showed to be suitable to simulate chemically heterogeneous environments to assess how contaminants disturb the habitat selection by organisms. In addition, it was evidenced how the contamination affects the organism's response to other ecological variables performing a cost-benefits analysis to reduce exposure to contamination and obtain more benefits. This novel methodology (nonforced exposure) and the integration of ecological interactions provide, respectively, new practical and conceptual approaches to assess the contamination effects from a landscape perspective. This could provide a little more of ecology to ecotoxicology studies.

#### **2.07.T-05 Single and Combined Effects of Pesticides and Metabolites in Microbial Litter Decomposition in Streams**

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The combined effects of pesticide parent compounds and their corresponding transformation products (metabolites) are often omitted in aquatic ecotoxicology studies. This gap of information is surprising given the large amount of pesticide molecules with relatively low half-life times and a plethora of metabolites found in aquatic environments. In the present study, we aim to compare the effect of tebuconazole and its metabolite hydroxy-tebuconazole on microbial litter decomposition, and to determine whether the presence of other pesticides and metabolites frequently found in surface waters (i.e., glyphosate and AMPA) increase the toxicity of the hydroxy-tebuconazole toward microbial litter decomposition. We conducted a microcosm experiment to address the research questions described above. A total of 15 glass aquariums of 15 L were filled with dechlorinated tap water supplemented with N and P and spiked with the different contaminants (i) tebuconazole (10 µg/L), ii) hydroxy-tebuconazole (10 µg/L), iii) glyphosate (0.1 µg/L) + aminomethylphosphonic acid (AMPA) (0.3 µg/L), and iv) the combination of hydroxy-tebuconazole (10 µg/L) + glyphosate (0.1 µg/L) + AMPA (0.3 µg/L) or without (v) control. Each aquarium contained precolonized black alder leaves enclosed in fine-mesh bags (0.5 mm mesh size) to assess microbial decomposition. The results show that tebuconazole and its metabolite hydroxy-tebuconazole had similar effects in reducing microbial decomposition of leaf litter and the diversity of aquatic hyphomycete communities, even if tebuconazole effects tended to be stronger than those of hydroxy-tebuconazole. Glyphosate and AMPA did not enhance the toxicity of hydroxy-tebuconazole on leaf-associated microbial communities, except when combining hydroxy-tebuconazole, glyphosate, and AMPA which consistently impaired the diversity of the aquatic hyphomycete communities.

#### **2.07.P Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part II: Microcosms, Mesocosms, Factorial Design, Community-Level)**

##### **2.07.P-Mo119 Aquatic Macroinvertebrates Under Multiple Stress: Insights from a Mesocosm Experiment**

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Freshwaters are frequently subject to a complex mixture of stressors like pollution and climate change. Moreover, wastewater effluents are one of the main sources of chemical pollutants in freshwater ecosystems. Multiple stressor impact on these ecosystems directly acts at the level of biodiversity-ecosystem functioning relationships. Studies on multiplicative effect of climate change, e.g., changing water temperature and chemical pollutants on freshwater biodiversity and implications thereof are limited. Accordingly, the aim of the current study was to investigate single and combined effects of wastewater effluent and elevated water temperature on aquatic macroinvertebrates. A mesocosm experiment was conducted with a simplified freshwater food web containing nonvascular macrophytes (moss) and Ephemeroptera, Plecoptera, Trichoptera, and Amphipoda, feeding as shredders and grazers. Samples were collected at the beginning and end of the experiment, whereas emerging animals were collected daily. Analyses enabling assessment of the response of nonmodel aquatic macroinvertebrates to selected stressors are ongoing, such as total protein and lipid content, and metabolome and lipidome profiling. Preliminary results show species-specific

response to stress. An increase in body weight of caddisfly larvae was observed at the end of experiment, except in multiple stressor treatment. Similarly, a drop in total lipid content in caddisfly larvae was related to increased water temperature or multiple stressor treatment. Furthermore, reduced lipid content related to multiple stressor treatment was also observed in adult mayflies. The current study will give novel insights into the molecular response of aquatic macroinvertebrates subjected to anthropogenic stressors.

#### **2.07.P-Mo120 The Impacts of Increased Temperature and Pollution on the Biological Functions of Two Caddisflies Species *Drusus croaticus* and *Allogamus uncatus***

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Aquatic ecosystems worldwide are increasingly exposed to both natural and anthropogenic stressors. Climate change-related alterations, like changes in temperature patterns of surface waters, are often linked with chemical pollution from different sources (e.g., surface runoff, discharge of both raw and treated wastewater, industrial waste disposal, etc.). Such a combination of stressors may have ecological and biological impacts on aquatic organisms such as freshwater insects which perform various important ecological functions in freshwater and terrestrial ecosystems. The main goals of the study were to: i) identify the potential impacts of multiple stressors (increased temperature and chemical contamination) on metabolomic and lipidomic profiles of two ecologically different species of caddisfly larvae, ii) analyze their behaviour expressed as locomotor activity (i.e., distance moved), and iii) evaluate the potential biological and ecological risk of climate change together with emerging contaminants. To simulate the environmental complexity of multiple stressors in laboratory conditions, we exposed caddisflies larvae *Drusus croaticus* and *Allogamus uncatus* separately to a cocktail of emerging contaminants in environmentally relevant concentrations of 500 ng, at optimal (8 °C) increased (12°C) temperature for 21 days. The cocktail included: i) psychoactive pharmaceuticals - fluoxetine, sertraline; ii) beta-blocker - sotalolol; iii) alkaloid - caffeine; iv) anticonvulsant - carbamazepine; v) antibiotic/macrolide - azithromycin; vi) industrial antioxidant - diphenylamine (DPA); vii) plasticizer - tributoxyl ethyl phosphate (TBEP), and viii) nonsteroidal anti-inflammatory drug ketoprofen. Preliminary results suggest both species of caddisflies showed decreased locomotor activity when exposed to a higher temperature (12 °C), before exposure to the cocktail of emerging contaminants. After exposure to the cocktail of emerging contaminants, both species exhibited differences in locomotor activity, most apparent on day 21: *D. croaticus* showed increased locomotor activity at 8 °C and decreased locomotor activity at 12 °C, while *A. uncatus* showed decreased locomotor activity at both temperatures compared to controls. Based on preliminary results of locomotor activity analyses, we concluded that multiple stressors may contribute to a disruption of natural behaviour, which could potentially lead to higher susceptibility of caddisflies to predation.

#### **2.07.P-Mo121 Combined Effects of Warming and Pesticides on Freshwater Invertebrates: A Mesocosm Study**

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Warming climate and chemical pollution are two widespread stressors of freshwater biota. Warming, predicted to be up to +8°C by 2100, can impact freshwater biota through a range of mechanisms such as increased individual growth rates and altered nutrient cycling. Pesticides are usually not intended for direct use in water bodies; however, they enter the aquatic compartment via spray drift, runoff, and leaching, are widespread in freshwater habitats, and usually reach surface waters as mixtures. Many freshwater organisms, unable to readily migrate between habitats when facing adverse conditions, are particularly threatened by these stressors.

We assessed the combined effects of warming and a mixture of pesticides on a freshwater community in a 2\*2 factorial design (i.e., with/without pesticides and with/without warming) in heated outdoor mesocosms. We combined a single-pulse cocktail of 6 pesticides (herbicides and fungicides), at concentrations commonly measured in surface waters in the Czech Republic, with warming at +4°C above ambient. The experimental community was assembled from common macroinvertebrate and plankton taxa from different trophic levels (e.g., predators, filter feeders and grazers, detritivores, and primary producers). The experiment ran during summer (June/August). We repeatedly measured pesticide concentrations, environmental parameters (dissolved oxygen, conductivity, pH, turbidity, and temperature), chlorophyll-*a* concentration, zooplankton density and aquatic insect emergence.

Both warming and pesticide mixture altered the invertebrate community composition and insect emergence patterns in the experiment. Pesticide mixture negatively affected the zooplankton dynamics and strongly inhibited the emergence of mayflies (*Cloeon* sp., Ephemeroptera), whereas both pesticides and warming had a strong negative effect on the emergence of the damselflies (Zygoptera). Our results suggest that environmentally relevant pesticide concentrations combined with climate warming can affect freshwater biota through individual-level responses leading to trophic cascades.

#### **2.07.P-Mo122 Disentangling the Effects of Multiple Stressors and Chemical Mixtures: Stressor Prioritization Using Monitoring Data Across Germany**

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River fauna and flora are exposed to a large number of micropollutants, which are released into the environment every day.

Combined effects of the complex chemical mixtures of these substances on community composition and diversity have been addressed in previous research studies. However, a variety of anthropogenic stressors including eutrophication, salinization, hydrological alteration and habitat degradation further influences aquatic biodiversity and related ecosystem functions of rivers. So far, effects of micropollutants have often been analyzed separately from those of other stressors and have only rarely been included in multiple-stressor studies. For a better guidance to river ecosystem management, comparative analyses of the relevance of individual stressors and combinations thereof are required.

We present the findings of a current study that aims at identifying and quantifying the effects of different stressors belonging to physicochemical, hydrological, and morphological stressors as well as risk variables representing the mixture toxicity for 51 selected micropollutants. The effects are evaluated for benthic invertebrates, benthic diatoms, and fishes, using a variety of ecological metrics and including biodiversity and ecosystem functions. A comprehensive dataset of rivers in Germany was compiled using available monitoring data taken in accordance with the EU Water Framework Directive. Random forests and subsequent variance partitioning were applied to put the different stressors' effects into a hierarchical context and to compare individual and combined effects on the three organism groups. At the same time, this study also highlights the challenging requirements for environmental data sets which are intended to be used for the analysis of multistressor effects and ultimately for the planning of appropriate management measures.

## **2.07.P-Mo123 Mediterranean Freshwater Ecosystems Under Pesticide and Climatic Stress: Effects of Imidacloprid, Elevated Temperatures, and Heatwaves**

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An increase in mean temperatures and higher severity and frequency of weather extremes, like heatwaves (HWs), are anticipated environmental changes in Europe under future global climate change (GCC), particularly in the Mediterranean. These climate alterations in daily temperature ranges and heat extremes will affect freshwater ecosystems and ectotherms that live within them. Because temperature per se may not only act as isolated perturbation, complex stressor interactions, like with pesticides, may lead to still unknown effects. Few multiple stressor studies investigated GCC-related temperature scenarios and chemical effects on population-, community-, or ecosystem-level. Among these studies, environmental realism regarding daily temperature fluctuations and thus natural variability are barely considered in experimental designs. Thus, our study goal was to investigate the multiple stressor effects of changing, elevated temperatures, HWs, and the neonicotinoid insecticide imidacloprid on Mediterranean freshwater ecosystems. We studied various ecosystem endpoints on different trophic levels. Multiple stressor effects on ecosystem processes and functioning were assessed by investigating stressor-induced alterations in community dynamics and structure, population dynamics, biomass dry-weight, and physicochemical water parameters. Finally, we built a structural equation model (SEM) to reveal direct and indirect food web effects and ecological effect chains.

Temperature was successfully controlled with the “Transportable temperature and heatwave control device” (TENTACLE) with an observed increase of the ambient temperature from April to July, ranging +15 to 30°C. Imidacloprid showed a temperature-dependent fate in water with the highest DT50-values under ambient temperatures whereas the lowest were noted under HWs. Zooplankton community analyses presented significantly 35% of the variation in species composition ( $p=0.02$ ) due to imidacloprid and temperature treatments. While imidacloprid as isolated stressor showed no significant community effects, significantly adverse effects of HWs in the presence and absence of imidacloprid were noted during the first two HWs followed by recovery dynamics. Individual population dynamics, such as of the rotifer *Polyartha* and Cyclopoids, presented significantly adverse effects. Besides direct effects, our SEM showed ecological effect chains through significantly negative relationships across trophic levels of the food web.

## **2.07.P-Mo124 Ecosystem Resilience and Recovery of a Natural Saline Lake in Response to a Supra-Seasonal Drought**

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Global climate change threatens aquatic ecosystems by altering rainfall patterns, air temperatures, and the severity and frequency of natural disasters such as floods and droughts. Natural saline lakes have great ecological importance but are overlooked in conservation efforts as their economic value is not well-understood. The primary threats to these natural saline lakes are climate- and anthropogenic-induced alterations to their hydrology and salinity, leading to modifications in their biodiversity and ecological functions. Lake Nyamithi is one of South Africa's only natural saline lakes and is threatened by legacy pollutants, climate change, regulation of water flow, and invasive species. Between 2015 and 2016, South Africa experienced one of the most severe droughts ever recorded and this study aimed to investigate the possible effects of the drought on the aquatic invertebrate and fish communities of Lake Nyamithi. Aquatic invertebrates and water samples were collected for biodiversity and water quality analyses respectively during pre-drought, peak drought, and recovery conditions while fishes and potential basal sources were collected during the predrought and recovery conditions. Various univariate and multivariate analytical techniques were used to assess aquatic invertebrate community structure and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of food web elements and fish were analysed using Bayesian mixing models and Bayesian Laymen metrics to investigate the fish community and the contribution of basal sources to



the fishes' diets. The salinity of the lake increased three-fold from the predrought ( $\pm 3 \text{ g.L}^{-1}$ ) to the drought ( $\pm 10 \text{ g.L}^{-1}$ ) with a consequent reduction in taxon richness of both aquatic invertebrates and fishes, including invasive species, as few taxa were able to tolerate the severely increased salinity. Several aquatic biota were able to (re)colonise the lake, excluding invasive species, once it received water and salinity decreased ( $< 8 \text{ g.L}^{-1}$ ). Although the food webs were similar between the pre-drought and recovery periods, the drought altered the basal food web from a  $C_3$  to  $C_4$ -dominated structure. Fish consumers adapted their diet accordingly by shifting their reliance from  $C_3$  to  $C_4$  basal sources. These findings demonstrate not only the environmental resilience of Lake Nyamithi and the biological plasticity of its aquatic biota but the importance of freshwater inflow to the continued functioning of natural saline lakes.

## **2.08 Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part III: Environmental Risk, Modelling, Ecosystem-Level)**

### **2.08.T-01 How a Data-Driven Stochastic Model Helps to Predict Micropollutant Discharges to Surface Waters**

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Urban wet-weather discharges (combined sewer overflows, CSO and stormwater outlets from separate sewers, SWO) contain various micropollutants which can negatively impact surface water quality. Recent work highlights that micropollutant discharges show a high spatiotemporal variability, which challenges the prediction of potentially toxic impacts to surface waters. Stochastic models based on known distributions can help in handling this inherent variability. We analyzed monitoring data from 77 CSO and SWO sites to estimate: i) the variability among sites and ii) predict micropollutant concentrations along a river with a stochastic model. The results show how we can make a first estimate of the impact of wet-weather discharges on receiving waters without site-specific micropollutant information, which is useful for regulators and utilities as a step toward zero pollution.

### **2.08.T-02 Integration of Climate Model Projections and Pesticide Application Scenarios for Probabilistic Risk Assessment with a Bayesian Network Model**

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We present a Northern European case study from the recent SETAC Pellston workshop on integration of global climate change (GCC) modeling into ecological risk assessment. In Northern Europe, global climate change (GCC) is expected to result in increased temperature and precipitation. The changes in weather patterns are expected to increase the occurrence of crop pests such as weeds, fungal disease, and insect pests. Increased pest pressures can in turn be expected to then alter agricultural practices such as the frequency and combination of pesticide applications. Additionally, climate change may potentially have more direct effects on the environmental exposures in the transport, fate, and degradation of pesticides.

A Bayesian network (BN) has previously been developed as a meta-model for incorporating future climate projections and pesticide application scenarios with information from toxic effects data to support environmental risk assessment for streams in agricultural areas. This BN model was initially parameterized for a Norwegian case study with predicted environmental concentrations from a processed-based pesticide exposure model and species sensitivity distributions derived from toxicity tests data.

Within the Pellston workshop, we aimed to improve the existing BN model by incorporating more recent and realistic climate change scenarios, a higher number of climate models, and better methods for regional downscaling. An exposure prediction model was run with alternative climate model that accounted for specific environmental factors linked to the study area, chemical properties of the pesticides, and more realistic pesticide application scenarios. Our experiences from this case study will aid efforts to better account for uncertainty related to climate change in exposure modeling, effect assessment, and risk characterization. The graphical display of the BN model approach can also aid communication of risk under climate change scenarios to stakeholders such as policy makers and regulators.

### **2.08.T-03 Chemical Pollution: An Overlooked Elephant in the Room of Ecology – Publication Patterns Reveal a Low Connection Between Chemical Pollution and Biodiversity Research**

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Argentina, (4)Functional Environmental Genomics, Senckenberg Biodiversity and Climate Research Centre, Germany, (5)Environment and Health, VU University, Amsterdam, Netherlands, (6)Goethe University Frankfurt, Frankfurt am Main, Germany, (7)Helmholtz Center for Environmental Research (UFZ), Leipzig, Germany, (8)Goethe University Frankfurt, Institute for Atmospheric and Environmental Sciences, Frankfurt am Main, Germany, (9)Goethe University Frankfurt, Institute of Physical Geography, Frankfurt am Main, Germany, (10)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, (11)Senckenberg Biodiversity and Climate Research Centre, Germany, (12)Department Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Frankfurt am Main, Germany, (13)Aquatic Ecotoxicology, Goethe University Frankfurt, Frankfurt am Main, Germany, (14)Integrative Parasitology and Zoophysiology, Goethe University Frankfurt, Frankfurt am Main, Germany, (15)LOEWE Centre for Translational Biodiversity Genomics, Frankfurt, Germany, (16)Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Frankfurt am Main, Germany, (17)Technical University of Darmstadt, Institute of History, History of the Middle Ages, Germany, (18)Institute of Biogeochemistry and Pollutant Dynamics, Switzerland, (19)Goethe University Frankfurt, Department of Human Geography, Frankfurt am Main, Germany, (20)ISOE- Institute for Social-Ecological Research, Germany, (21)Geisenheim University, Department of Crop Protection, Germany; and Instituto de Bio y Geociencias del Noroeste Argentino (IBIGEO-CONICET) and Universidad Nacional de Salta, Argentina, Geisenheim, Germany, (22)Section River Ecosystem Management, Senckenberg Society for Nature Research, Germany, (23)Faculty of Biological Sciences, Goethe University Frankfurt; and Senckenberg Society for Nature Research, Frankfurt am Main, Germany, (24)Goethe University Frankfurt, Department of Law, Frankfurt am Main, Germany, (25)Justus Liebig University of Giessen, Institute for Insect Biotechnology, Germany, (26)Faculty of Biological Sciences, Goethe University Frankfurt; and ISOE - Institute for Social-Ecological Research, Frankfurt am Main, Germany Since the onset of the Anthropocene, anthropogenic drivers including changes in land use, climate change, invasion of alien species, and chemical pollution have played a central role in the degradation of ecosystems and loss of biodiversity globally.

In the case of chemical pollution, six decades of ecotoxicological research have yielded tens of thousands of scientific papers. In order to analyze general research and publication patterns on chemical pollution, we conducted two Scopus searches. The first search used several synonyms of "chemical pollution" to target papers published since 1992. The second search (ecological search) imposed variants of the terms "ecology" or "ecosystem" to the title, abstract, or keywords to capture studies that were related to ecology or ecosystems. We compared these results with similar searches for other drivers and biodiversity loss, and found that while drivers such as climate change and habitat loss have been readily embraced by general ecological research, chemical pollution remains surprisingly under-assessed and hence remains as an overlooked elephant in the room of ecology. Detailed inspection of the results of the ecological search showed that a large proportion of the studies on chemical pollution lack sufficient experimental, field, or analysis complexity that are necessary to enable derivation of true ecological implications. Instead, many papers seem to have mentioned ecology or ecosystems merely to frame the study within a wider context.

Chemical pollution papers have been published in a narrow selection of specialised journals that rarely publish articles on other topics. Few ecological journals are in this set. This shows that, while research on chemical pollution is very prolific, it has thus far been conducted using a single-discipline approach and predominantly in disconnection from general ecology. The rich extensive scientific literature base, methodological arsenal developed during decades of ecotoxicological research, and increasing political and international commitment on the topic provide a powerful platform to promote a higher degree of interdisciplinarity in chemical pollution research. This is pressingly needed to deliver science that better informs political and regulatory action for the protection of Earth's ecosystems and biodiversity.

## **2.08.T-04 How Will Climate Change Affect the Distribution of Macroinvertebrates in European Surface Waters and their Sensitivity to Chemical Pollution?**

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Climate change is predicted to have profound impacts on freshwater ecosystems due to rising temperatures and altered hydrological regimes. Particularly, extreme events have been shown to deeply influence freshwater biota, but how they will influence the distribution of freshwater organisms at the European scale remains an open question. Using an ensemble of bioclimatic envelope models (BEMs) considering future climatic and hydrological changes, we modelled the climatic suitability of 286 stream macroinvertebrate genera across Europe for 2050 and 2080 using a spatial resolution of 5 arc minutes. We first studied how macroinvertebrate taxa will shift their distribution and how this is reflected in terms of taxa gains/losses across Europe. Then, we used biological traits to assess whether the changes in taxa distribution are mirrored by shifts in functional richness. Finally, we analyzed how the relative portion of sensitive taxa to different chemical groups (i.e., narcotics, acetylcholinesterase (AChE) inhibitors, ion/osmoregulatory/circulatory (IOC) impairment chemicals, neurotoxicants, reactive substances, and electron transport inhibitors) will shift due to climate change using taxonomy-based and biological trait-sensitivity models. We found that climate change will result in a loss of suitable habitats for a large part of the modelled taxa in the southern and eastern parts of Europe, while a general increase in habitat suitability is expected in the Northern part of the continent. Yet, these large changes in habitat suitability did not result on marked changes in functional richness in our analysis, suggesting that functional redundancy will play a significant role in the delivery of ecosystem functions in the future. Finally, changes in the relative distribution of sensitive taxa will strongly depend on the chemical group considered. For most chemical groups, the changes in relative number of sensitive taxa were only moderate (<10%). However, two chemical groups (i.e., AChE

and IOC impairment) show strong differences between the present and the future scenarios. This study shows that abiotic factors related to global climate change can influence distribution patterns of freshwater macroinvertebrates and the relative sensitivity of macroinvertebrate species assemblages to chemical exposure. This suggests that water quality monitoring tools and risk assessment should consider the variation in species distribution related to global climate change.

#### **2.08.T-05 Climate Change and Pesticides Century-Long Impact on Freshwater Functional Biodiversity**

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Biodiversity supports diverse ecosystem functions and services, yet is threatened globally by anthropogenic environmental change such as chemical pollution, climate change and land use change.

These abiotic factors occur and interact over decade-to-century long timescales and the effects are therefore difficult to study directly. Furthermore, shorter term studies begin from an already shifted baseline of community assemblage and may miss longer term trends. Here, we have used a sedimentary archive from a lake with a well-known history of chemical pollution and climate change spanning the past 100 years. We have reconstructed the community-wide responses to environmental change using multimarker sedDNA metabarcoding and reconstructed functional ecosystem changes using KEGG pathways reconstructed from the lake bacterial community.

We study 100 years of community-level biodiversity, climate change and chemical pollution trends. We apply explainable network models with multimodal learning to community-level functional biodiversity, to establish correlations with biocides and climate change records. We identify the top individual abiotic factors and combinations of chemical and climate factors with adverse effects on functional biodiversity. The freshwater community assemblage and functionality changed over time without returning to its original state, even if the lake partially recovered in recent times. Insecticides and fungicides, combined with extreme temperature events and precipitations, explained up to 90% of the functional biodiversity changes. Community-level biodiversity reliably explained freshwater ecosystem shifts whereas traditional quality indices and indicator species proved to be poor metrics for these shifts. The ranking of individual chemicals enables the identification of priority targets for regulation of chemicals with adverse effects on biodiversity.

#### **2.08.P Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part III: Environnemental Risk, Modelling, Ecosystem-Level)**

##### **2.08.P-Mo125 Emissions of Organic Micropollutants and Antibiotic-Resistant Bacteria via Different Urban Entry Pathways**

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Micropollutants as well as pathogens including antibiotic-resistant bacteria (ARB) and antibiotic resistant genes (ARG) can enter surface waters via wastewater treatment plants (WWTP). However, also, combined sewer overflows (CSO) and rain sewers can be relevant entry pathways for these contaminants and might gain increasing importance due to an expected higher frequency of heavy rain events as a result of climate change. Thus, the aim of this study is to assess the relevance of different entry pathways of pollutants, ARB and ARG. A monitoring concept was designed and applied in Koblenz (Germany) allowing for the event-based, simultaneous sampling and analysis of organic micropollutants, metals as well as ARB and ARG. The sampling strategy includes i) flow-proportional 24-hours composite samples over the course of one week at a WWTP effluent and ii) automatic event-based, flow-proportional sampling at a CSO and a rain sewer. About 70 organic micropollutants are analyzed in the dissolved and particle phase using multi-target methods by liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS). Furthermore, metals and polyaromatic hydrocarbons (PAHs) are analyzed according to ISO guidelines. ARG and ARB are determined i) qualitatively by selective agar plates and antibiograms, and ii) quantitatively by qPCR and modern full length-16S sequencing. Results from first sampling campaigns of a WWTP effluent and a CSO event show clear differences in pollutant concentrations as well as microbial communities and presence of ARB and ARG. In the WWTP effluent, higher concentrations of organic micropollutants associated with domestic wastewater were detected with the exception of compounds with a high degradation potential (e.g., ibuprofen) and compounds associated with outdoor applications (e.g., mecoprop). In the CSO sample, higher concentrations of PAHs and metals as well as a higher bacterial burden were detected. Pollutant loads were generally higher in the WWTP effluent. Still, the CSO represents a considerable and for some pollutants and pathogens an exclusive emission source. The monitoring will be extended to additional monitoring sites, non-target analysis as well as further events and seasons allowing for a comprehensive characterisation and assessment of different entry pathways and evaluation of correlations among the occurrence of pollutants and antibiotic resistance.

##### **2.08.P-Mo126 Measured Peak Concentrations of Organic Micropollutants during Combined Sewer Overflows**

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Combined sewer overflows (CSOs) are a pathway of wastewater and stormwater into open water bodies, where they can have toxicological effects on the aquatic environment. Combined sewage is a mixture of a large number of organic micropollutants

(MPs) applied either indoors or outdoors with strongly fluctuating concentrations due to variation in use patterns at the source and dilution. Previous studies showed that the toxicity of fluctuating concentrations can be higher than their average and that the toxicity can depend on the sequence in which the substances occur. Furthermore, a mixture can be toxic even though the single substances lie below the toxicity thresholds. Hence, a comprehensive risk assessment requires the consideration of the complete mixture of MPs at high temporal resolution. However, most studies measuring MPs in CSOs analysed event mean concentrations. Therefore, we aim to quantify the concentrations of a large set of organic MPs (300 targets) in CSOs at high temporal resolution as input for a more comprehensive toxicity analysis and to identify discharge mitigation measures.

For this, we sampled and analysed polar organic MPs at two CSO sites in Switzerland. At the first site, we measured three overflow events with a field deployable mass spectrometer delivering 20 minutes resolution data. For seven more events at site 1 and 10 events at site 2 we used automated samplers and LC-HRMS analysis in the lab, to generate 10 minutes composite samples (based on 2 min grab samples). The samples were analysed for 300 target substances representing different sources, out of which 63 were evaluated so far.

The results show that the organic MP concentrations can vary strongly during an overflow event. This is especially true for small catchments and substances coming from sources with high variation, such as pharmaceuticals. Furthermore, we could find 51 out of 63 target substances so far, which indicates that combined sewage consists of many different organic micropollutants with diverse temporal profiles.

This analysis delivers a unique dataset showing the sequence and concentration levels of peaks for a large number of organic MPs at CSOs. The results will serve as a realistic exposure scenario and help to design more realistic toxicity assessments.

## **2.08.P-Mo127 Pathways of Biocides in an Exemplary Urban Catchment: Impact of Combined Sewer Overflows and Storm Water Outlets on Surface Water Pollution**

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Biocides have a widespread use in urban areas (e.g., disinfectants, material preservatives, and pest control products) and can enter the environment through multiple pathways. In urban areas the sewer system is the main entry pathway for a large number of substances from different sources like households, industry, and road runoff. Substances, among them biocides, are often discharged directly into surface waters via storm water outlets and combined sewer overflows without any treatment. Numerous studies show that these substances can frequently be detected and therefore influence surface water quality. However, the discharge of biocides into surface waters via effluents of sewage treatment plants (STP) and combined sewer overflows (CSO) is little investigated. Therefore, the German Environment Agency (UBA) initiated a project to investigate the impact of different urban entry paths on river water contamination with biocides in an exemplary urban catchment in Germany. For this study a number of sampling sites were selected in the city of Karlsruhe to examine the river Alb, the effluent of the municipal wastewater treatment plant, storm water outlets, and combined sewer overflows. Based on criteria like physicochemical properties, ecotoxicity, and relevance for surface water pollution a total of 42 biocidal active substances and transformation products (mainly disinfectants, material preservatives, and pest control products) were prioritized and will be analyzed in about 130 samples over a period of a year. Preliminary results show that about 26 biocides and transformation products were detected in a large number of samples. Mainly biocides used as material preservatives, e.g., carbendazim, diuron, and terbutryn, were found in nearly all samples of the different sampling sites, whereas other substances, for example the pyrethroid permethrin was mostly found in CSO samples. Furthermore, to assess the relevance for surface water contamination the exceedances of the environmental quality standards (EQS) or the predicted no effect concentrations (PNEC) were examined in this project and show exceedances, e.g., for the substances imidacloprid (STP effluent) and permethrin (CSO). The project provided important insights into the occurrence of biocides in urban runoff components and identified combined sewer overflows as a relevant emission pathway in urban areas.

## **2.08.P-Mo128 Comparative Assessment of the Ecotoxicity of Rainwater Runoff Originating from a Highway and a Low-Traffic Zone**

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Rainwater runoff is receiving increased attention as a potential vector of inorganic and organic pollution toward surface water. In particular, concerns are being raised about the impact of transported heavy metals and transformation products from tire wear particles. Furthermore, climate change could potentially alter this impact, especially in regions where it will manifest as more intense precipitation events combined with longer dry periods. For the purpose of this explorative assessment, runoff samples at a highway junction and a low-traffic residential area were taken in the vicinity of Ghent, Belgium. Each sample consisted of 5 consecutive subsamples, with the purpose to investigate the existence of a so-called first-flush effect. Organics present in the subsamples were extracted via a mildly hydrophobic Chromabond HR-X Solid Phase Extraction (SPE) resin, and metals concentrations were determined via Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) measurements. Additionally, the toxicity of the extracted organic compounds was assessed using a 72-hour algae growth inhibition assay using *Raphidocelis subcapitata*. The generated data were used to compare the two sample locations as well as to assess the existence of a first-flush effect. Finally, further research pathways and assessment refinement options are discussed.

## **2.08.P-Mo129 Molecular Tool Shows Effects of Extreme Weather Events in Aquatic Quality Monitoring**

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The Netherlands is a delta of a total of four international rivers. Rijkswaterstaat is responsible for the aquatic quality in the large rivers, lakes, and the coastal areas.

Rijkswaterstaat has started a pilot to assess the applicability of Next Generation Sequencing (NGS) to monitor the effects of chemicals and weather on the aquatic ecosystem as well as the resilience of the aquatic system in case of incidents and extreme weather conditions. The pilot is being performed at the border laboratory in the Meuse. Each month a water sample is taken and analyzed by means of NGS. This characterizes all known bacteria and archaea and gives a clear overview of the biodiversity in the river. The biodiversity is compared with the results of the chemical analyses and the flow-through bioassays that are regularly performed at the border laboratory as well as climatic conditions. During the pilot we will gain insight into the annual fluctuations of the biodiversity. The pilot has been running for over 1.5 years now. During this period we have had some extreme weather events: summer flooding in July 2021 and extreme drought in July and August 2022. This resulted in changed conditions in the river. The microbial populations have been changed as a result of this, showing that the microbial population is not only influenced by chemical conditions but also by (extreme) weather conditions.

## **2.08.P-Mo130 A Bayesian Network Approach to Assess the Impacts of Climate Change and the 'Farm-to-Fork' Strategy on the Ecological Risks of Pesticides in a Protected Mediterranean Wetland**

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Pollution by agricultural pesticides is one of the most important pressures affecting Mediterranean coastal wetlands. Pesticide risks are expected to be influenced by climate change, which will result in an increase of temperatures and a decrease in annual precipitation in these areas. These future climate prospects could also increase the prevalence of agricultural pests, which may result in an increase of pesticide dosages by farmers. On the other hand, the European 'Farm-to-Fork' strategy promotes the 50% reduction of pesticide usage by 2030. The influence of climate change and pesticide management measures on pesticide risks need to be evaluated making use of realistic environmental scenarios. This study aimed to assess how different climate change and pesticide use scenarios affect the ecological risks of pesticides in the Albufera Natural Park, a protected Mediterranean coastal wetland. We performed a probabilistic risk assessment for nine pesticides applied in rice production using scenarios comprised of three climatic regimes (the 2008 record and projections for 2050 and 2100), three pesticide application regimes (the recommended dose, and 50% increase and 50% decrease), and their combinations. The scenarios were used to simulate pesticide exposure concentrations in the water column of the rice paddies using the RICEWQ model. The pesticide effects were assessed using acute and chronic Species Sensitivity Distributions (SSDs) built with laboratory toxicity data for aquatic organisms. Risk Quotients were calculated as probability distributions using a Bayesian network approach, using best fitting distributions for the calculated exposure data and SSDs. Our results show that future climate projections will influence exposure concentrations for some of the studied pesticides, yielding higher dissipation and lower exposure and risks in scenarios dominated by an increase of temperatures. Management measures such as those dictated by the European 'Farm-to-Fork' strategy are crucial to reduce risks in Mediterranean coastal wetlands but will need additional protection measures. Our case study shows that pesticides such as azoxystrobin, difenoconazole and MCPA are posing high ecological risks and should be further monitored in Mediterranean coastal wetlands surrounded by intensive rice farming.

## **2.08.P-Mo131 Climate Change Impacts in Estarreja Region – The TERRA Project**

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Over the last years, great efforts have been made to tackle the climate crisis; however, climate change continues to impact our planet. According to the Intergovernmental Panel on Climate Change (IPCC) predictions, atmospheric CO<sub>2</sub> levels and mean temperature are expected to rise continuously, inducing an increase in the frequency, intensity, and duration of extreme weather events (e.g., droughts and floods). The impact of climate change may be exacerbated in contaminated areas, like those in proximity to industrial chemical complexes, where the ecosystems might be under long-term multiple pressures. The Industrial Chemical Complex in Estarreja, in northern Portugal, has been operating for more than half-century and is one example of a historically contaminated area. Considering the reported increase of worldwide extreme climate event frequency in the last years, the project TERRA aims at understanding the effects of climate change alterations on terrestrial and aquatic ecosystems using as a case study the contaminated soils area in Estarreja. For that, extreme climate events, considering climate change scenarios predicted by IPCC (including changes in combined climate factors), will be simulated to evaluate their effects on the ecotoxicity in terrestrial and aquatic organisms. Moreover, soil/water properties (e.g., pH, chemical content, soil organic carbon) will be evaluated to establish the link between soil/water characteristics and potential ecotoxicological effects under changing climate factors. Additionally, TERRA intends to understand the dynamics behind the contamination in the Estarreja region by evaluating contamination levels in this area and comparing with previous studies conducted more than five years ago. TERRA will provide crucial data for Portugal and the world regarding the consequences of climate change in contaminated areas. The present project

will also promote easy and active communication with society, with the final aim of increasing awareness about climate change and its environmental impacts.

#### **2.08.P-Mo132 Integrating Expertise on Climate Modeling and Environmental Risk Assessment: A SETAC Pellston Workshop in the Oslo Fjord**

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A SETAC Pellston workshop was organized 20-24 June 2022 near Oslo, Norway, with the topic "Incorporating climate change model predictions into ecological risk assessments to help inform risk management, adaptation, and mitigation strategies".

SETAC Pellston workshops in general aim to bring together environmental professionals from academia, government, industry/business, and NGOs to advance the state of knowledge and identify solutions for pressing environmental challenges. The goals of this workshop were:

1. To bring leading practitioners in global climate change (GCC) science and ecological risk assessment together to catalyze and solidify future workings across the two communities.
2. Develop methods for incorporating GCC science into ecological risk assessment frameworks to ensure that they address current and potential future risks, whether for chemicals or other stressors.
3. Communicate the conclusions to key stakeholders including the IPCC, national and international governmental organizations, and researchers to establish SETAC as a key partner in GCC science.

An important purpose of this workshop was thus to involve expertise on climate modeling as well as expertise on chemistry, toxicology, and environmental risk assessment. Approximately 20 scientists were invited to the workshop to provide a balance of expertise as well as sectors, geographical locations, genders, and career stages. The workshop location Oscarsborg is a historical fortress located on a small island in the Oslo fjord, selected in line with the principle of holding SETAC Pellston workshops in secluded locations to encourage focused attention from participants. Nevertheless, real-time online participation was offered as an alternative to in-person attendance when necessary. This poster will focus on the workshop organisation and expertise, while the scientific outcome of the workshop will be reported in other presentations in this session.

#### **2.08.V Ecosystems Responses Under a Multiple Stressors Scenario in a Rapidly Changing Climate (Part III: Environnemental Risk, Modelling, Ecosystem-Level)**

##### **2.08.V-01 Climate Change and Atmospheric Deposition as Drivers of Forest Ecosystem Integrity and Services: A Modelling Approach Crossing Different Spatial Scales**

**Stefan Nickel<sup>1</sup>**, **Winfried Schröder<sup>1,2</sup>** and **Martin Jenssen<sup>3</sup>**, (1)Planwerk, Germany, (2)Faculty II, University of Vechta, Chair of Landscape Ecology, Germany, (3)Evangelische Johanniter-Schulen Wriezen, Wriezen, Germany

Action 5 of the EU Biodiversity Strategy calls Member States to map and assess the condition of ecosystems and their services. Therefore, we developed a spatial explicit methodology for assessing and classifying forest ecosystem integrity under conditions of climate change and nitrogen deposition. The methodology presented covers three spatial levels: the nation-wide level (Germany), the regional (exemplified by the Kellerwald National Parc) and the site level in terms of forest stands representing forest types. These were derived from vegetation and soil data covering about 22 000 forest stands. Based on this, the methodology enables to determine whether the conditions of forest ecosystem types differ between that in a reference period (1960-1990) from current (1991-2010) and potential future ecosystems conditions (2011-2070). The deviances of ecosystem integrity indicators from the reference states were quantified on an ordinal scale from very low to very high enabling to estimate at three spatial levels whether an ecosystem type will get extinct and replaced by another one or not. The first step investigated at the national level whether the spatial pattern of these forest types is expected to be stable over time compared to the reference period or whether spatial shifts and extinction of forest types are likely to occur. This analysis indicated, that up to 2070 the areal coverage of forest ecosystem types at montane and altimontane levels will decrease while that of ecosystem types in Atlantic and sub-Atlantic ecoregions will increase. Respective statistics were computed for the EUNIS and FFH ecosystem classification as well as for threatened plant species and their biotopes. Using 14 indicators for 6 ecosystem functions, historical reference conditions were quantified for 60 forest ecosystem types. The comparison with current and potential future ecosystem conditions enabled the ordinal scaled classification of ecosystem integrity at the level of indicators, functions, and ecosystem types. Projections of expected ecosystem changes were based on: 1. Dynamic modelling of soil indicators considering climate change and anthropogenic nitrogen deposition at the site level; 2. Fuzzy modelling and mapping soil moisture; and 3. Rule-based classification and GIS-Mapping of ecosystem services.

#### **2.09 Ecotoxicology of Biota Inhabiting Wetland Ecosystems**

##### **2.09.P-We072 Microplastic Contamination of Water and Tadpoles in Amphibian Breeding Pools in Scotland (UK)**

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Microplastic (MP) contamination has been widely reported across a range of aquatic environments and their compartments including in water, sediments, and biota. There are indications that small freshwaters (SFWs) such as ponds may be highly

contaminated with MPs due to their low volumes combined with close proximity to MP sources, such as domestic wastewater inflows, urban run-off, and agricultural land. Amphibians are one of the most common vertebrates to inhabit SFWs. In this study, we first investigated MP contamination of amphibian breeding pools *via* water, sediment, and internal levels (common toad [*Bufo bufo*] tadpoles). We then analysed for relationships between MP abundance and surrounding land use characteristics (Geographic Information systems approach) to identify possible sources of contamination. To characterise land use, we used QGIS (3.28) using a buffer zone of 0.5 miles around the shoreline of each pond, and associations between land types and MP contamination levels were analysed (Generalised Linear Mixed Modelling). Finally, we analysed for correlations (Spearman Rank correlation) between internal MP levels and tadpole development (body mass, length). Water and sediment samples were collected from nine ponds across the central belt of Scotland at four-time points in Spring 2021. Where found ( $n = 4$  ponds), 20 tadpoles were collected *per* pond on the third and fourth visits. The levels of MPs found across all site characterisations ranged between 0–30 items/L in water and 12–1484 items/kg in sediments. Tadpoles also contained MPs ranging between 0 – 163 items/g tissue (0 – 19 items/individual). MP abundance in sediments was positively correlated with the proportion of land cover characterised as industrial ( $R^2 = 0.037$ ;  $p = 0.002$ ) or as pasture ( $R^2 = 0.024$ ;  $p = 0.011$ ). In tadpoles, the vast majority of MPs were found in the gut (81.26 %,  $n = 464$ ) compared to the liver (8.06 %,  $n = 46$ ), and carcass (10.68 %,  $n = 61$ ), suggesting dietary uptake. Across all sites, a significant negative correlation ( $p < 0.01$ ) was found between tadpole MP abundance and body mass ( $R^2 = -0.1135$ ), total length ( $R^2 = -0.1587$ ) and snout-vent length ( $R^2 = -0.1805$ ), suggesting that MPs may be negatively impacting wild tadpole health. These results provide important insights into the extent of MP pollution in SFWs, which currently do not form part of routine water quality monitoring, as well as potential links between MP ingestion and health endpoints in wild amphibians.

## 2.09.P-We073 Impact of Pesticide Overspray on Amphibians and the use of Earthworms as Surrogates for Evaluating its Effects

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Amphibians are the most threatened group of vertebrates, and the impact of pesticides has been pointed as one of their main threats. However, amphibians are not generally included in the environmental risk assessment of pesticides, assuming that the evaluation conducted on other vertebrate taxa grants them protection. This assumption raises concern, considering the differences in structure and function of amphibian skin with respect to the integument of other groups. Here, we studied the effects of overspray with the current-use fungicide oxathiapiprolin on juvenile frogs (*Pelophylax perezi*) and in adult earthworms (*Eisenia andrei*), with the aim of evaluating their suitability as a surrogate invertebrate species for amphibians and therefore promote the reduction and replacement of vertebrate use in ecotoxicity experimentation. To do so, froglets and earthworms were grown in the laboratory and exposed by overspray using a pump calibrated to simulate realistic pesticide application regimes. Amphibian assays included two individuals per replicate that were processed on days 7 and 21 postexposure for histological examination, whereas earthworm assays were run for 72 hours. We also recorded survivorship and changes in body condition. In amphibians, mortality was higher in the oxathiapiprolin-treated groups than in the negative control group, but the differences were not significant. Besides, a reduction in the body condition of frogs exposed to oxathiapiprolin was noted relative to negative control frogs on day 7. On the other hand, exposure to oxathiapiprolin caused no mortality in adult earthworms and no effects on their weight. Regarding effects on the tissues, the analysis of H&E-stained histological sections from frogs on day 7 postexposure showed subdermal interstitial tissues of an intense black resembling melanin accumulation and enlarged pigmented macrophage centres. This marked pigmentation could be a physiological response to damage directly or indirectly produced by oxathiapiprolin. Furthermore, exposure to oxathiapiprolin caused macroscopic lesions in the skin of earthworms. The fact that there is an effect in the tissues after pesticide overspray in both organisms suggests that earthworms could be fitting as surrogates for amphibians regarding dermal toxicity. The results add valuable data on the toxicity of current-use pesticides on amphibians.

## 2.09.P-We077 Water Quality and Land-Use Impacts on Development of Larval Amphibians in the UK

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When last assessed in 2004, 32% of amphibian species were threatened with extinction, and at least 43% were in decline, which is faster than any other vertebrate group. As the vast majority of amphibians incorporate an aquatic larval phase during their lifecycle in mainly small freshwater bodies (i.e., ponds, pools, ditches, streams), water quality and the surrounding land use of the water body have importance for their development. As early life stages are known to be particularly sensitive to various stressors compared to their adult counterparts, conditions during aquatic stages are fundamental to both their immediate health and subsequent adult fitness. The small size, high spatial density, and tendency to complete development within a small water body also make larval amphibians excellent models for assessing environmental stressor impacts on animal health. In this study, we collected body length and/or body mass and developmental stage data for wild tadpoles from two anuran species (common toad: *Bufo bufo*; common frog: *Rana temporaria*) and a newt species (palmate or smooth newt: *Lissotriton* sp.) over several years (2015, 2019, 2021, 2022) across Devon and the central belt of Scotland (UK) along with pond water quality parameters (temperature, pH, total dissolved solids, conductivity, salinity) and made assessments of the surrounding land using a Geographic Imaging Systems (GIS) approach. As expected, over the pre-metamorphic development period, there was a positive correlation

between snout-vent length (SVL)/body mass and developmental stage in all species/sampling years; however, the strength of this association differed between years and species ( $R^2$  range: 0.42-0.91). Data were further analysed using a series of generalised linear mixed models with SVL/body mass data were normalised by developmental stage prior to statistical analysis. For common frogs (2015, 2021), higher pH (range: 5.7-8.2) was associated with larger body mass. A similar effect was observed for common toads (2019, 2022), whereby higher pH (range: 5.2-7.8) was associated with larger SVL. However, for common frogs/newts (2022), no relationship was found between pH (range: 5.6-8.1) and SVL. Further analyses of water quality and surrounding land are being undertaken to understand the relative influence of these parameters on amphibian larval development. The resulting data will also provide an important baseline for studies using morphology for wild UK larval amphibian populations.

## **2.09.T-01 Effects of the Fungicide Azoxystrobin in Two Ecotypes Representative of Mediterranean Coastal Wetlands: A Mesocosm Experiment**

**Pablo Amador<sup>1</sup>**, Constanza Vega Olivares<sup>2</sup>, Natividad Isabel Navarro<sup>1</sup>, Jesús Moratalla<sup>1</sup>, Melina Crettaz<sup>2</sup>, Daniel Franco<sup>2</sup> and Andreu Rico<sup>1</sup>, (1)Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain, (2)IMDEA Water Institute, Spain

Coastal wetlands are under intense anthropogenic pressure, which affects the structure and functioning of their communities. In Spain, the emission of nutrients and pesticides from intensive agriculture have been considered as the main drivers of biodiversity loss. Using a mesocosm experiment, this study aims to study the sensitivity of two different communities representative of the Albufera Natural Park (the Albufera Lake and the surrounding springs, called *Ullals*) to the fungicide Azoxystrobin (applied as Ortiva, YF9246). Effects were evaluated over a period of 56 days on different structural parameters (phytoplankton, zooplankton, macroinvertebrates, insect emergence, macrophyte growth) and functional parameters (organic matter decomposition, biofilm growth). Fungicide effects on the structure of the distinct communities were assessed using the Principal Response Curve method, while effects on single populations and other functional and water quality parameters were estimated using the Williams test and calculating No-Observed-Effect Concentrations (NOEC) for each model ecosystem. Results show that both communities respond in a different manner to the fungicide application. Macrophyte growth (*Ceratophyllum*) was affected from the 2 µg/L concentration on in the Albufera treatment but no significant differences were found for Ullals. Effect's timespan also varied between treatments, with periphyton colonization rate being affected from day 0 in Albufera and from day 28 in the Ullal treatment. Invertebrates also showed a different response depending on the treatment. Even though zooplankton biomass was significantly higher in the *Albufera* treatments than in the *Ullal* treatments, both systems shared a large drop in the abundance of the *Ceriodaphnia*. A very clear trend was found in macroinvertebrates within the *Ullal* treatment, where the crustaceans *Echinogammarus*, *Palaemonetes*, and *Dugastella* disappeared completely in the 200 µg/L concentration. In general, we conclude that current exposure levels may affect the structure and functioning of Mediterranean coastal wetland ecosystems. However, the Ullal communities seem to be more sensitive to azoxystrobin contamination, potentially due to differences in chemical bioavailability and species composition. This study highlights the need to prioritise these habitats for conservation and the need to set specific water quality standards for particular ecotypes within the Mediterranean region.

## **2.09.T-02 Using Nonlethal Measures of Fish and Invertebrate Health to Assess the Impacts of Fertilizing Wild Rice with Aquaculture Waste**

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The growth of freshwater finfish aquaculture is impaired by inefficiencies associated with the transportation of feed from coastal areas to production sites. Large volumes of wastewater, enriched with nitrogen and phosphorus, can also contribute to eutrophication, threatening the health of downstream aquatic ecosystems. Wetland plants and algae can remove nutrients and can then be harvested and incorporated into functional fish feeds, reducing requirements to transport feed. The ability of wild rice (*Zizania palustris* L.) to assimilate nutrients was evaluated in mesocosms (2 m diameter; ~1500 L) amended with a gradient of aquaculture waste applied over 12 weeks. Reference enclosures, not receiving waste, were also included in the study. Aqueous concentrations of total phosphorus, total dissolved phosphorus, and soluble reactive phosphorus increased with volumes of aquaculture waste applied and throughout the growing season. Ecosystem health was evaluated in each mesocosm by examining zooplankton taxon abundance and diversity metrics, using traditional enumeration techniques and eDNA, and compared to reference enclosures that did not receive waste applications. Fathead minnows were also stocked into each mesocosm. No differences were observed in their body condition, liver, or gonad size at the end of the study. Mucus was sampled from these fish twice over the growing season to examine transcriptomic and proteomic responses to elevated nutrient concentrations in water. Measures of ecosystem health and water quality will be used to establish the capacity of cultured wild rice to remove nutrients from aquaculture waste streams in a co-cultured scenario. Future field-based studies will also explore the diversion of excess nutrients, not removed by wild rice, to produce algae and floating vegetation that can be incorporated into functional fish feeds.

## **2.09.T-03 Synergistic Effects of Larval Exposure to an Aquatic Pollutant and Food Stress Get Stronger After Metamorphosis in an Amphibious Insect of Temporary Wetlands**

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Many animals living in temporary wetlands have a complex life cycle with aquatic larvae and terrestrial adults, as seen for example in amphibians and amphibious (semiaquatic) insects such as damselflies. To improve the ecological risk assessment of aquatic pollutants it is needed to study their effects not only in the aquatic larval stage, but also in the terrestrial adult stage of the



many animals with a complex life cycle. This remains understudied, especially with regard to interactive effects between aquatic pollutants and natural abiotic stressors. We studied effects of exposure to the pesticide DNP (2,4-Dinitrophenol) and how these were modulated by limited food availability in the aquatic larvae, and the possible delayed effects in the terrestrial adults of an amphibious insect specialized in temporary wetlands: the damselfly *Lestes viridis*. Our results revealed that DNP and low food each had negative effects on the life history, behaviour, and to a lesser extent on the physiology of not only the larvae, but also the adults. The negative effect on larval development time resulted in delayed metamorphosis which may have strong fitness consequences by increasing mortality from pond drying in temporary wetlands. Food limitation magnified the toxic effects of DNP as seen by a strong decline in larval survival, metamorphosis success, and adult lifespan. Notably, the synergism between the aquatic pollutant and food limitation for survival-related traits was stronger in the nonexposed adults than in the exposed larvae, likely because metamorphosis is stressful itself. Our results highlight that identifying effects of aquatic pollutants in temporary wetlands and synergisms with natural abiotic stressors, not only in the aquatic larval but also in the terrestrial adult stage, is crucial to fully assess the ecological impact of aquatic pollutants and to reveal the impact on the receiving terrestrial ecosystem through a changed aquatic-terrestrial subsidy.

## 2.09.T-04 Predicting Amphibian Body Burdens After Dermal Uptake of Pesticides from Soil

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Although currently considered covered by surrogate bird and mammal Environmental Risk Assessment (ERA) schemes, concerns for amphibian risk assessment have arisen regarding dermal exposure to pesticides in the terrestrial phase, primarily due to the high permeability of their skin and the lack of a protective layer such as fur or feathers. It is therefore hypothesized that dermal uptake could significantly surpass oral/dietary exposure. It thus becomes necessary to determine the relative importance of different exposure routes for the integrated toxicity outcome, including dermal uptake of contaminants for terrestrial amphibians.

To this end, a modelling approach with the aim of providing realistic estimates of amphibian body burdens after dermal exposure to contaminated soil was developed and is here presented.

A one-compartment Toxicokinetic (TK) model was derived and tested using a publicly available dataset containing relevant exposure and uptake information for juvenile frogs exposed to 12 different pesticides. Parameters used in the model included tested species, body weight, soil concentration, body burdens, skin surface area, skin thickness,  $K_{oc}$ , permeability coefficient of the pesticide, and water uptake rate of the animals. Additionally, Poly-parameter Linear Free Energy Relationships modelling was used to describe the distribution of the corresponding pesticide between water and frog tissue and integrated into the equation.

Modelled body burdens were compared to actual, measured body burdens for a total of 407 individuals exposed to the 12 different pesticides. In order to compare the overall predictability of the model for a given pesticide, predictions and measured concentrations were averaged over all individuals for a given pesticide.

Overall, a good concordance between modelled and measured body burdens was observed, with the predicted and measured body burdens differing by a factor of 3 on average. Results were subsequently plotted on a log-log plot and a regression analysis performed, resulting in an  $R^2$  of 0.78 and a correlation coefficient of 0.88, suggesting good predictivity of the model.

Based on these results, we suggest this or similar models could be used to characterize dermal exposure in amphibians, to screen for pesticides of concern and prioritize ERA efforts, while at the same time reducing the need for animal testing.

## 2.09.P Ecotoxicology of Biota Inhabiting Wetland Ecosystems

### 2.09.P-We072 Microplastic Contamination of Water and Tadpoles in Amphibian Breeding Pools in Scotland (UK)

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Microplastic (MP) contamination has been widely reported across a range of aquatic environments and their compartments including in water, sediments, and biota. There are indications that small freshwaters (SFWs) such as ponds may be highly contaminated with MPs due to their low volumes combined with close proximity to MP sources, such as domestic wastewater inflows, urban run-off, and agricultural land. Amphibians are one of the most common vertebrates to inhabit SFWs. In this study, we first investigated MP contamination of amphibian breeding pools *via* water, sediment, and internal levels (common toad [*Bufo bufo*] tadpoles). We then analysed for relationships between MP abundance and surrounding land use characteristics (Geographic Information systems approach) to identify possible sources of contamination. To characterise land use, we used QGIS (3.28) using a buffer zone of 0.5 miles around the shoreline of each pond, and associations between land types and MP contamination levels were analysed (Generalised Linear Mixed Modelling). Finally, we analysed for correlations (Spearman Rank correlation) between internal MP levels and tadpole development (body mass, length). Water and sediment samples were collected from nine ponds across the central belt of Scotland at four-time points in Spring 2021. Where found ( $n = 4$  ponds), 20 tadpoles were collected *per* pond on the third and fourth visits. The levels of MPs found across all site characterisations ranged between 0–30 items/L in water and 12–1484 items/kg in sediments. Tadpoles also contained MPs ranging between 0 – 163 items/g tissue (0 – 19 items/individual). MP abundance in sediments was positively correlated with the proportion of land cover characterised as industrial ( $R^2 = 0.037$ ;  $p = 0.002$ ) or as pasture ( $R^2 = 0.024$ ;  $p = 0.011$ ). In tadpoles, the vast majority of MPs were found in the

gut (81.26 %,  $n = 464$ ) compared to the liver (8.06 %,  $n = 46$ ), and carcass (10.68 %,  $n = 61$ ), suggesting dietary uptake. Across all sites, a significant negative correlation ( $p < 0.01$ ) was found between tadpole MP abundance and body mass ( $R^2 = -0.1135$ ), total length ( $R^2 = -0.1587$ ) and snout-vent length ( $R^2 = -0.1805$ ), suggesting that MPs may be negatively impacting wild tadpole health. These results provide important insights into the extent of MP pollution in SFWs, which currently do not form part of routine water quality monitoring, as well as potential links between MP ingestion and health endpoints in wild amphibians.

### **2.09.P-We075 Third Update of the Ecotoxicology of Amphibians and Reptiles Textbook - Recent Advancements in Amphibian and Reptile Ecotoxicology**

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Prior to the year 2000, ecotoxicological research focused on amphibians or reptiles was scarce compared to that for other vertebrate groups, often consisting of basic experimental approaches with limited ecological relevance. The First World Congress of Herpetology held in 1989 brought about by reports of global amphibian population declines, dramatically highlighted the importance and potential fate of this class of organisms. The 21st century has seen an increased understanding of ecotoxicology of amphibians and reptiles. More research has yielded greater numbers and diversity of publications, plus two editions of a textbook, *Ecotoxicology of Amphibians and Reptiles* by Sparling et al., which SETAC's Interest Group members are now seeking to update. For the second edition, Sparling et al. performed a literature review covering the years 1996-2008 showing that 10 years after publication of the first edition, amphibians and reptiles remained underrepresented in the ecotoxicology literature relative to other vertebrates. Since the second edition, there has been a dramatic increase in the number of research studies and papers focusing on amphibians and reptiles and the effects and burdens of contaminants on these two classes of vertebrates. Therefore, we conducted a title-based literature review for the years 2006-2022. Although the number of citations increased since the second edition, preliminary results show that amphibians and reptiles still remain underrepresented in ecotoxicology literature. Of the 22 056 citations examined (1.3-fold increase in comparison to 1996-2008), 71% were for fish, 11% for mammals, and 10% for birds. Since the second edition, the number of citations related to amphibians increased from 3.8% to 5.3%. For reptiles, the number of citations increased from 0.8% to 3.1%. Moreover, there was a 2- and 5-fold increase of contaminant-related publications for amphibians and reptiles, respectively. Further evaluations on topics such as main contaminant classes, annual publication numbers, most studied taxa and emerging stressors such as climate change and diseases, are ongoing and detailed results will be presented at the conference.

### **2.09.P-We076 Occurrence of Common Frog and Common Toad in Agricultural Fields in Germany: Potential for Exposure to Plant Protection Products**

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The appropriate and effective assessment of the ecological risk of chemicals to amphibians is a current topic of debate. Amphibians are a highly endangered class of vertebrates and while the major causes for population declines are habitat loss and diseases, other factors like introduced predatory species and exposure to chemicals, including plant protection products (PPPs), are also implied. For risk assessment of PPPs, a consideration of exposure and toxicity is required. Here, we present results of a project that studied occurrence and habitat use of adult and metamorph common frogs (*Rana temporaria*) and common toads (*Bufo bufo*) in an agricultural landscape, and the implications for potential exposure to PPPs.

The 4 sites (ponds) selected for the study had >85% agricultural land (mainly cereal fields) surrounding the ponds and were suitable to study amphibian occurrence in agricultural land. Adults were monitored with radio tracking devices for 2 years and juveniles were caught in pitfall traps using drift fences and then released.

Adult frogs and toads were rarely observed in arable fields, i.e., only 0.5% and 4% of the sightings, respectively. Abundance of metamorphs in arable land around two sites was 0% and 16% of the catches. For a third site, completely surrounded by arable fields, the number was higher at 39% (frogs) and 26% (toads) of sightings. The fourth site had no interpretable metamorph data, because of an accidental manure contamination of the water body killing most tadpoles. Metamorphs of both species tended to remain close to their natal ponds and their in-field occurrence decreased with distance from the water body.

Thus, the presence of amphibians in arable fields was limited and primarily occurred later in the summer, when metamorphs had left the breeding pond and when crops were denser and hence crop interception of sprayed PPPs is higher. This, together with the hiding behaviour during the day would limit the risk of dermal exposure.

The use of buffer zones around water bodies in agricultural areas would thus be highly effective as a risk mitigation strategy in protecting the metamorph life stages and will also reduce spray drift entry into water bodies following PPP applications. We hope that the results will contribute to data-driven environmental decision-making and to the discussion on effective risk assessment and mitigation options for this taxonomic group.

### **2.09.P-We078 Characterizing the Effects of Chronic Conventional Heavy Crude Oil Exposure on the Behaviour of Larval Wood Frog (*Lithobates sylvaticus*) in Outdoor Mesocosms**

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Amphibians are amongst the most globally endangered groups of organisms, in part due to the sensitivity of their habitats. A large portion of North American crude oil reserves are landlocked and their exploitation requires extensive networks of pipelines, rails, and roads which in many cases cross sensitive amphibian habitats, such as wetlands. As part of a larger program of research aimed to understand the fate, impacts, and remediation approaches to oil spills in boreal freshwater ecosystems, the Floating Wetland Treatments to Enhance Remediation (FloWTER) study examined the efficacy of plant-microbe relationships for *in situ* degradation of oil-derived hydrocarbons in outdoor mesocosms. As part of the study, the uptake and effects of *in situ* chronic exposure to a conventional heavy crude oil (CHV) water-accommodated fraction on the growth, development, and behaviour of wood frog (*Lithobates sylvaticus*) tadpoles were characterized in the presence or absence of floating aquatic plants, and a no oil control. Weekly behavioural assays were recorded on a subset of five tadpoles from each mesocosm to examine swim speed, latency time, distance traveled, and nearest neighbour distance over the exposure period. Additional tests assessing changes in the response of the tadpoles to a predator cue were performed at the end of the exposure period. The sensitivity of the behavioural test set-up and the wood frog tadpoles themselves was also assessed by performing complete concentration-response test with anxiolytic and anxiogenic reference chemicals (ethanol and caffeine), a step that to the best of our knowledge, had not been previously reported for this species. No significant treatment effects were observed in the basal behaviour of the tadpoles. The presence of the predator cue induced an increase in activity in the tadpoles; however, we did not observe differences in the ability of the tadpoles to respond to the cue between treatments. Our reference chemical tests did show that our test system was able to detect changes in behaviour in wood frog tadpoles. The observed lack of treatment effects was likely due to the observed rapid dissipation of the oil-derived hydrocarbons from the water column resulting in limited exposure to the tadpoles. This research provides much needed data on the toxicological effects of CHV on amphibians to inform risk assessment related to the over-land transportation of crude oil and potential impacts of freshwater oil spills

## **2.09.P-We079 Occurrence and Potential Ecotoxicity of Pesticides Transformation Products in Pond Ecosystem**

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In Europe, numerous small water bodies (SWB) punctuate the hydrographic network without being integrated in the Water Framework Directive (WFD), making these areas understudied. Ponds are an integral part of SWB and highly valuable wetlands, providing ecosystem and economic services. Due to their proximity to agricultural lands, their contamination statuses are subjected to change, especially regarding the occurrence of Plant Protection Products (PPPs).

The newly frequent detection of PPPs and some of their transformation products (TPs) in aquatic ecosystems, such as ponds, led to the inclusion of these substances in the monitoring of contaminants in French surface water bodies. Indeed, the potential ecotoxicity of these TPs has newly raised questions and aroused widespread interest concerning the risk that they can pose to wildlife inhabiting these areas.

Our project first aims to generate data on TPs occurrence in different ponds and then attempts to identify some associated potential ecotoxicological effects. To do so, *in vivo* assays were realized by characterizing different biological responses of freshwater crustaceans exposed to previously identified TPs. Identification of TPs by chemical analysis in 12 sampling sites (i.e., ponds) is shown. The main work presented here tries to establish a link between data concerning ecotoxicological values of TPs, generated from *in silico* analyses (QSAR prediction), and those obtained by employing *in vivo* models.

Here, a focus is placed on the species *Daphnia magna* by applying the OECD 202 standard test as a first approach. After the exposure, the TPs studied such as 2-chloronicotinic acid or metolachlor CGA 357704 show different toxicities among them and compared to their parent compounds (e.g., boscalid and S-metolachlor). This provides new data on the acute toxicity of TPs for primary consumers such as water fleas. Our results suggest that these substances should be considered when assessing the ecotoxicity of ponds water.

Considering different combinations of substances quantified *in situ* (in ponds) and most frequently detected ones, cocktails of PPPs and TPs will also be tested on daphnia. The freshwater algae *Raphidocelis subcapitata* will be also employed to be more relevant with the type of TPs found, mainly deriving from herbicides (e.g., chloroacetanilides).

## **2.09.P-We081 Morphological Temporal Effect of Metallic Nanoparticle Contamination from Environmental Atmospheric Particulate Matter in Gills of the Mangrove Crab *Ucides cordatus***

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The atmospheric particulate matters (PM) are usually related to mining and metallurgical industry. Since the Industrial Revolution, the contamination by metals is still a problem and tracking the contaminant in the environment and the biota can be hard work. In water, metallic PM contaminants appear to dissociate into nanoparticles, so the presence in circulatory system may facilitate their internalization into cells. The effect of metallic PM with non- and emerging metals in estuarine biota is still up for characterization. Therefore, this study aimed to describe the histopathological effects in mangrove crab gills under atmospheric PM. The animals were acclimatized for a period of 10 days at DCM/UNIFESP (Santos, SP). Then, there were 30 days of exposure in 40 L aquariums in quintuplicates, consisting of the following groups: control (CTL); concentration 0.01 g.L<sup>-1</sup> (1); 0.1g.L<sup>-1</sup> (2); and 1.0 g.L<sup>-1</sup> (3). Each aquarium contained 10 individuals under constant aeration and photoperiod of

12Hlight:12Hdark. Tissue collections were defined by the times: 7 days after exposure (T7); 15 days (T15) and 30 days (T30). The atmospheric MP were from Complex Tubarão, Espírito Santo, Brazil. The tissues were fixed in formaline 10% and proceeded under histological routine. Cuts of 5 µm were stained with Hematoxylin-Eosin (HE). In T7, the gills were, in general, well-structured and intact. Only in concentration 3, the pillar cells presented nucleus with the dysmorphic chromatin. In T15, both the pillar cells and the coticle capsule were unstructured, with major presence of hemocytes and immune cells in the lumen of the lamellae and hemal channel, in all concentrations. These characteristics can be a hemorrhagic process leading to an inflammatory and necrosis. So, in T30, the gills were completed unstructured, they had unidentifiable cells given the damage caused. In conclusion, after an exposure of 30 days, the gill structures were damaged by time exposition, whatever the concentration. The atmospheric PM can morphological responses that after a long exposure time can lead to chronic diseases and death, resulting in populational impact and affecting lots of niches.

#### **2.09.P-We082 Heavy Metal Body Burdens and their Interaction with Metabolites in the Pacific Black Duck (*Anas Superciliosa*) in Victoria, Australia**

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Heavy metals are cumulative toxicants with important implications for wildlife in freshwater systems as their accumulation has the potential to cause negative health and ecological effects. Over the past decade, the study of metabolomics has expanded in multiple scientific fields such as environmental science, and toxicology and its application have recently increased in the humanities and medical sciences where mounting evidence has shown that metabolites are related to exposure to heavy metals. However, comparable studies on animal species especially ducks are currently lacking. This study aimed to examine the body burden of heavy metals and metabolites in Pacific black ducks from three Victorian freshwater biomes in Australia: one peri-urban site in south-west Victoria [designated Lake Connewarre (LC)] and two sites in rural coastal eastern Victoria [designated Macleod Morass (MM) and Dowd Morass (DM)] and assess whether the change of metabolites was correlated with high levels of heavy metal exposure. Feathers were used to analyse chromium, copper, iron, mercury, manganese, lead, and zinc while muscles were analysed for metabolites. Inductively coupled plasma mass spectrometry and gas chromatography-mass spectrometry were used to analyse the metals and metabolites, respectively. Principal Component Analysis was conducted to determine whether the change of these metabolites was linked to heavy metals. The overall results within each location showed that the mean concentration of all metals was lower in MM than in the other locations LC and MM. Lead and mercury were both significantly different between LC and MM, but showed no difference between DM and MM. Aminoimidazole, α-lactic acid, lysine, taurine, and linoleic acid metabolites were significantly different between LC and MM. However, their change was not linked with lead or mercury indicating that the non-invasive feather analysis was not directly correlated with changes in metabolites. It is possible that the excretion of these toxic metals into feathers is effective as a detoxification mechanism, hence not linked to metabolic function. This study provides toxicology information with ecological and health implications in waterbirds from a relatively poorly studied global region in south-eastern mainland Australia.

#### **2.09.P-We083 Lindane and Endosulfan Exposure in White-Headed Duck and Marbled Teal from a Wetland Dedicated to Agricultural Irrigation: Tracking the Success of Pesticides Ban in El Hondo Natural Reserve (SE Spain)**

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El Hondo Nature Reserve collects water from the River Vinalopó and the River Segura, and is comprised of two large regulating reservoirs (1100 ha.) and their associated wetlands. The wetland was created in the 1920s to provide water for irrigation to local farms. Other relevant human activities include hunting and fishing. This wetland appears in the Ramsar list, and it is also classified as Protected Area for Birds due to not only the abundance of birds (172 species, 50 of them breeding there) but also the ecological relevance of some bird species, such as marbled teal (*Marmaronetta angustirostris*) and white-headed duck (*Oxyura leucocephala*).

Although the agricultural use of lindane and endosulfan was banned in Europe around 2007, there is a particular need for the ongoing monitoring of these banned organochlorine pesticides, and measures should be taken to mitigate the exposure of vulnerable populations. One hundred and fourteen ducks found dead in El Hondo between 1998-2022 were selected and classified in "before"/"after" 2007 (date of death). Liver and brain of 56 marbled teals (41/15) and 58 white-headed ducks (26/26) were analyzed. After extraction and purification, one microliter of the final extract was analyzed using a GC/MS. The identification and quantification of the compounds was based on an external standard prepared by dissolving in hexane a reference standard: HCH isomers (α-HCH, β-HCH, γ-HCH (lindane) and δ-HCH) and endosulfan (endosulfan I, endosulfan II, endosulfan sulfate).

No differences in pesticide concentrations were observed between species. In general, frequency of detection and concentrations of lindane and endosulfan were significantly lower in ducks dead after pesticide prohibition. Lindane, together β-HCH, was the HCH isomer mainly detected in both species, with concentrations in liver higher than in brain. Endosulfan I was more detected (70-80%) than endosulfan II (36-44%) in both species in the period before pesticide prohibition; however, after 2007, the frequency of detection of both compounds decreased until 20-30%.

In conclusion, these results show that lindane and endosulfan exposure in anatidae inhabiting El Hondo has decreased significantly, which suggests a lower use of these compounds since their prohibition. However, they are still being detected in some animals, which could indicate an illegal market or the existence of commercial product remnants in some farms.

## 2.10.A Field Studies, Monitoring and Effects Research in Wildlife

### 2.10.A.T-01 POPs Maternal Transference and Consequences for Egg Development and Reproduction Success in Green Sea Turtles (*Chelonia mydas*)

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Persistent Organic Pollutants (POPs) have global distribution and are known to be harmful to wildlife. Due to their complex life cycle, their diets and migratory behaviours, marine turtles tend to accumulate these pollutants in their tissues. Just a few studies have been made to understand the load and the POP effects in sea turtles' tissues that may influence their overall health and especially the quality of their eggs to infer about the health of future sea turtles' generations. Five of the seven existing and threatened sea turtle species can be found in São Tomé and Príncipe archipelago. The green sea turtle, *Chelonia mydas*, is the most common species nesting in the archipelago, and there are some indications about possible effects of metals on nesting females in those islands. However, effects of POPs in these females or in their eggs are still lacking. Thus, the aim of this study was to determine the levels of different POPs (PCBs, OCPs and PBDEs), both in the blood of nesting females of green sea turtles and in their eggs, to infer about maternal transference, and to evaluate if such contamination could be associated with differences in the morphology, composition, and quality of their eggs, as well as reproductive success (clutch size, hatching rate, and emergence rate). For sea turtle eggs, most of the egg mass is represented by the yolk, which is highly rich in lipids. As metabolic and signalling mediators, yolk fatty acids (FAs) are essential for embryo development and posthatch activity. The results indicate that the screened contaminants significantly affected the egg compartments and lipid reserves. FA results from the egg yolk showed that the ones linked to the polar fraction (mostly associated to lipoproteins) seemed to be more influenced by the POP levels. A maternal transference phenomenon was also elucidated here for most of the contaminant's congeners analysed. This study provided valuable information about the current POP levels in female green sea turtles and in their eggs from São Tomé Island, indicating their widespread presence. Moreover, this study showed how the contaminants transferred from the female turtles to their eggs may be affecting their reproduction by altering egg properties for proper embryo development, their clutch size and nesting season success. Further studies to monitor marine pollution and damages derived from it in other developing stages and marine turtle species are needed to increase conservation efforts.

### 2.10.A.T-02 Anticoagulant Rodenticide Toxicity in Terrestrial Raptors: New Tools to Estimate the Potential Impact of Mortality on Populations

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Anticoagulant rodenticides (ARs) have caused widespread contamination and poisoning of predators and scavengers, particularly terrestrial birds of prey. AR poisoning is diagnosed mainly on evidence of hemorrhage in conjunction with summed hepatic AR residues > 0.1 – 0.2 ug/g liver wet weight. However, simple summation of residues is confounded by variability in the potency and toxicokinetics of individual residues. Other factors also affect sensitivity of nontargets to ARs, including: exposure dose, timing and frequency of exposure, and intra- and interspecific differences in sensitivity related to body condition, nutritional state, and other factors. There is, therefore, a need for compound and species-specific AR toxicity criteria to assess population-level effects on predatory birds. Previously we developed a novel approach based on probabilistic methods. However, with limited datasets and number of species, those assessments were limited in determining uncertainty around the probabilistic curves. Other shortcomings included: assumed additivity, inability to analyze individual species and compounds, uneven sample size in the binary data set of birds classified as 1s (positive) or 0s (negative) for pathophysiological signs of anticoagulant rodenticide poisoning, as well as an assessment of how trauma cases bias results. To improve the approach, we compiled a database of hepatic SGAR residues and postmortem evaluations from 951 terrestrial raptor carcasses collected throughout Canada and the USA, 1989 – 2021, representing 26 species of mainly owls and hawks. We developed species- and residue-specific probability curves to assess the toxicity of bromadiolone, brodifacoum, and difethialone and adjusted total SGARs using toxic equivalency factors from individual residue curves. To address unequal sampling of 0s and 1s in the data, logistic regressions were run on Monte-Carlo simulated binary data.

### 2.10.A.T-03 Development of Innovative Physiological-Based Kinetic (PBK) Model to Predict Exposure of Small Mammals to Anticoagulant Rodenticides, Posing High Risks to Environmental Health (Urine and/or Faeces)

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Anticoagulant rodenticides (ARs) are widely used in the world. ARs can inactivate the vitamin K clotting system, potentially leading to bleeding episodes and even the death of the exposed organisms. However, this clotting system is not exclusive for rodents, indicating that ARs may cause high potential risks to nontarget species.

Hence, a specific monitoring system to quantify the exposure of nontarget organisms to ARs and assess associated risks is required. Physiologically based kinetic (PBK) modelling and PBK-based reverse dosimetry are proposed to underpin nondestructive monitoring approaches. In this study we have developed such PBK models for ARs to be used to quantify actual exposure of target species to ARs by measuring the concentration of parent compounds and associated metabolites in, e.g., faeces and urine. Parameters describing the kinetics of ARs in target species were quantified through *in vitro* experiments and available *in vivo* exposure data and literature. Overall, the results reveal that predicted time-dependent changes of ARs matched the data from available *in vivo* studies quite well. Based on this, further PBK modelling-based reverse dosimetry methods will be refined to predict the *in vivo* levels of ARs in rodents. In the models, the concentration of ARs in both liver and blood increased to highest level within 24 hours and then slowly decreased. Hydroxylated metabolites of ARs were detected in the metabolism assay and will be further used to optimize the model interpretations and perform risk assessments of ARs.

#### **2.10.A.T-04 Environmental Pollution and Nutritional Quality Modulate Immune Response of the Wood Mouse (*Apodemus sylvaticus*) through Hormonal Disturbances**

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Over the last decades, rapid changes in our societies introduced multiple anthropogenic stressors such as pollution or low diet quality/availability related to habitat loss and fragmentation. Diet quality was found to be dampened by these changes reducing the availability of micronutrients in the diet. Among them, selenium (Se) is an essential element, involved in metabolic processes (including immunity) and reactive oxygen species scavenging. In opposition, nonessential elements like cadmium (Cd) and lead (Pb) can enhance physiological disturbances on health in wildlife. Both Cd and Pb enhance immune cell damage and decrease cellular immunity, promoting higher susceptibility to infectious diseases. This study aimed at evaluating the ability of Cd and Pb and low nutritional quality to modulate immune response to a lipopolysaccharide (LPS) challenge in wood mice (*Apodemus sylvaticus*) under potential control of endocrine regulation. Wood mice were trapped in sites exhibiting “High” and “Low” levels of Cd and Pb contamination near a former smelter in Northern France (Metaleurop Nord). Individuals were either challenged immediately after capture or maintained 5 days in captivity, fed standard or Se-deficient diet. Immune response to challenge was measured with leukocyte count and plasma concentration of TNF- $\alpha$ , a pro-inflammatory cytokine involved in the recruitment of immune cells. Faecal and plasma corticosterone (CORT), a stress-hormone involved in murine anti-inflammatory processes, was measured to assess potential endocrine mechanisms. Hepatic Cd, Pb and Se concentrations were also determined. Higher hepatic Se and lower faecal CORT were measured in free-ranging wood mice from “High” site. The immune response of LPS-challenged individuals from “High” site displayed lower levels of white blood cells count and a higher plasma TNF- $\alpha$  concentration than in “Low” site. Animals maintained in captivity for 5 days had lower concentrations of plasma TNF- $\alpha$ . Individuals from “High” site maintained in captivity had lower levels of inflammation and no increase of anti-inflammatory CORT when fed with Se-deficient diet. These results are in favour of a higher inflammatory response to challenge in free-ranging animals exposed to Cd and Pb. Mechanisms by which inflammatory response is modulated may be due to neuroendocrine disturbances mediated by Cd and/or Pb exposure. The role of Se on the immune response to challenge and its environmental sources remain to be determined.

#### **2.10.A.T-05 In Natura Exposure of Soil, Earthworms, and Small Mammals to Pesticides in Agricultural Landscapes: From Fears to Facts**

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A significant lack of knowledge on the fate of pesticides in the environment and their unintended effects hinders the understanding and mitigation of their impacts on biodiversity. As part of the RESCAPE project (RESistance of agricultural landSCAPEs to pesticide transfers in soils and living organisms) the exposure of soil, earthworms, and small wild mammals (that can represent a trophic chain) to currently used pesticides and “old” pesticides (banned and restricted in use) was studied to shed light on the knowledge on these issues. Multiresidue pesticide analyses revealed that 100% of the soils, 92% of the earthworms and 100% of the small mammals contained at least one pesticide. In small mammals, a total of 112 compounds (fungicides, herbicides, and insecticides) were detected, with 32-65 residues detected per individual (13-26 old pesticides and 18-41 currently used pesticides in each animal). For earthworms, 46% of samples (coming from both treated fields and nontreated habitats) presented a high risk of chronic toxicity. Moreover, among the currently used pesticides, some were systematically found in the three matrices: the insecticide imidacloprid, the herbicide diflufenican, and the fungicide epoxiconazole. This work suggests that exposure of wildlife to mixtures of pesticides is a rule rather than an exception, highlighting the need to consider the concept of the eco-exposome and questioning the relevance of current assessment and evaluation processes.

#### **2.10.B Field Studies, Monitoring and Effects Research in Wildlife**

### 2.10.B.T-01 Factors Modulating Exposure of Red-Legged Partridges to Pesticides: Diet and Spatial Ecology

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The treatment of seeds with pesticides is an extended practice in current agriculture. Granivorous birds, such as the red-legged partridge (*Alectoris rufa*), have a high exposure risk as they can consume those seeds that remain on the surface during sowing. Experimental studies have evidenced effects on the reproductive capacity of red-legged partridges fed with seeds treated with approved doses of triazole fungicides, the active ingredients most commonly used for treatment of nonirrigated cereal seeds. However, information about the exposure risk for wild birds and the factors that modulate it is still scarce. The aim of this study is to know how the uptake of triazole fungicides by partridges is influenced either by the consumption of cultivated plant species during the sowing season or by the presence of birds on recently sown fields. We also studied if the spatial ecology of red-legged partridges can serve as a proxy to estimate their pesticide risk exposure. From autumn 2017 to summer 2018, we collected feces (n = 144) from 35 different partridge flocks in an agricultural area in central Spain. We analyzed pesticide residues in feces by LC-MS and diet composition by metabarcoding. We tracked another 15 birds using GPS loggers that recorded daily positions in order to monitor the use of different patches. Residues of tebuconazole were detected in 16.6% of birds. During the peak of the sowing season, cultivated plants constituted more than half (56.1%) of the diet ingested by partridges, with barley as the most consumed species (40.7%). Spatial ecology revealed that all flocks where tebuconazole was detected had recently used sown fields, while negative flocks had no contact with these areas. We found that the study of spatial ecology in partridges could be a useful tool for estimating exposure risk after sowing. Our results highlight the importance of considering detailed ecological information for the refinement of the exposure characterization as part of the environmental risk assessment of pesticides.

### 2.10.B.T-02 Feathers as Temporal Archives of Ecological Stress and Metal Exposure in a Terrestrial Raptor: A Long-Term Study on Breeding Tawny Owls

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Metal contamination is a global environmental problem. As exposure of wildlife to heavy metals such as mercury has been associated to adverse biological effects, mitigation regulatory efforts have been implemented. Long-term studies are therefore well suited to investigate the adverse effects of toxic metals since they span the large variability of metal environmental contamination while also integrating fluctuating environmental conditions such as climate and food availability. However, long-term studies that investigate multiple ecological and environmental stressors on the stress and fitness of wildlife are challenging and therefore scarce. To assess the impact of metal pollution on wildlife, raptors have often been used as biomonitors due to their high position in food webs and high exposure to contaminants. Here, we studied a resident nest box population of tawny owls (*Strix aluco*) in Central Norway over a 34-year period. The tawny owl is a common terrestrial bird of prey in Europe and a long-lived species that is territorial throughout the year. Between 1986 and 2019, population data and tail feathers from females have been collected annually resulting in approximately 1200 feathers. Their feathers represent an excellent nondestructive matrix to retrospectively analyze metal contamination in their local environment and stable isotopes as dietary proxies. In addition, corticosterone (CORT) levels in feathers were measured. As the primary avian glucocorticoid, feather CORT provides a long-term integrated measurement of physiological stress in relation to ecological variables including reproductive and fitness related traits or climate and food availability. In addition, the stress of being contaminated has been a subject of growing interest, and previous studies have also reported links between feather CORT and metals in wild birds. In the current study, we aimed to examine how annual variations in anthropogenic (metals contamination) and natural environmental stressors (climate variables, and diet), individually or jointly, modulate the individual physiological stress response (CORT) and fitness (reproduction and survival) in a key Norwegian terrestrial sentinel species. Finally, we investigated the ecological drivers of CORT, and examined whether CORT could be a proximal mechanism underlying the effects of stressors on the breeding success and fitness of tawny owls living in a rapidly changing environment and exposed to multiple stressors.

### 2.10.B.T-03 Monitoring Veterinary Pharmaceuticals in Livestock Carcasses Supplemented for Avian Scavengers

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Veterinary pharmaceuticals are considered emerging contaminants in the environment. Some of these compounds reach avian scavengers through trophic chain when consuming contaminated carcasses. This is of increasing concern in EU, especially after the detection of the first case of diclofenac intoxication in one cinereous vulture (*Aegypius monachus*) in Spain. Here we analysed 48 commonly used veterinary pharmaceuticals in tissues from livestock carcasses supplied at feeding stations for avian scavengers consumption in Spain. These included antibiotics, nonsteroidal antiinflammatory drugs (NSAIDs), external antiparasitic drugs, and one antimuscarinic drug. Carcasses were collected in 2022 in 10 feeding sites in Aragon. Samples selected were liver, muscle, and kidney from pig (n = 93), sheep (n = 18), goat (n = 7), cow (n = 1), chicken (n = 30), and rabbit (n = 10). These were analysed using liquid chromatography coupled to a mass spectrometry (LC-MS-TQ). NSAIDs were detected in 7.5% (12/159) and antibiotics in 53.5% (85/159) of the total livestock carcasses supplied. Diclofenac was detected in 1.1%, ketoprofen in 3.2%, flunixin in 1.1%, hydroxyethyl salicylate 1.1%, and meloxicam in 6.5% of the analysed pig tissues. Some of these

compounds were also detected in sheep (flunixin, 16.7%), goat (flunixin, 14.3%), and chicken (flunixin, 13.3%; meloxicam: 6.7%). Antibiotics detected in the total of carcasses analysed were: ciprofloxacin and lincomycin, both 17.6%; tilmicosin, 16.4%; marbofloxacin, 10.1%; enrofloxacin, 9.4%; tulathromycin, 5.7%; trimethoprim; 4.4%, sulfadiazine, 3.8%; gamithromycin, 1.9%; doxycycline, sulfadimidine, and oxytetracycline, all 1.3%; tetracycline, chloramphenicol, and florfenicol, all 0.6%. Highly toxic NSAIDs for vultures, including diclofenac, were detected in livestock carcasses. Even if most of the detected NSAIDs were not present at high doses that could potentially cause mortality, the highest flunixin levels detected could already pose a risk to vultures. Pharmaceuticals were mostly detected in pig carcasses, including highly toxic NSAIDs, suggesting a higher risk of exposure through their consumption. For the first time we present data on veterinary pharmaceutical residues in carrion from chicken and rabbit, which show high prevalence of antibiotics. The obtained information will be used to describe the determinants of the exposure to pharmaceuticals in avian scavengers necessary to establish measures of risk mitigation.

#### **2.10.B.T-04 The ATTAC Guiding Principles to Openly and Collaboratively Share Wildlife Ecotoxicology Data**

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The inability to quantitatively integrate scattered data regarding potential threats posed by the increasing total amount and diversity of chemical substances in our environment limits our ability to understand whether existing regulations and management actions sufficiently protect wildlife. Systematic literature reviews and meta-analyses are great scientific tools to build upon the current push for accessibility under the Open Science and FAIR movements. Despite the potential of such integrative analyses, the emergence of innovative findings in wildlife ecology and ecotoxicology is still too rare relative to the potential that is hidden within the entirety of the available scattered data. To promote the reuse of wildlife ecotoxicology data, we propose the ATTAC workflow which comprises five key steps (Access, Transparency, Transferability, Add-ons, and Conservation sensitivity) along the chain of collecting, homogenizing, and integrating data for subsequent meta-analyses. The ATTAC workflow brings together guidelines supporting both the data prime movers and re-users. As such, the ATTAC workflow could promote an open and collaborative wildlife ecotoxicology able to reach a major objective in this applied field, namely, providing strong scientific support for regulations and management actions to protect and preserve wildlife species in the context of chemical risk. This presentation will provide a brief overview of the ATTAC workflow, focusing on the five main steps, the philosophy behind, and its added value to promote an open and collaborative wildlife ecotoxicology in the coming years.

#### **2.10.B.T-05 Frontiers in Quantifying Wildlife Behavioural Responses to Chemical Pollution**

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Animal behaviour is remarkably sensitive to disruption by chemical pollution, with widespread implications for ecological and evolutionary processes in contaminated wildlife populations. However, conventional approaches applied to study the impacts of chemical pollutants on wildlife behaviour seldom address the complexity of natural environments in which contamination occurs. This talk will present a new review by Bertram et al. (2022, Biological Reviews, <https://doi.org/10.1111/brv.12844>), which aims to guide the rapidly developing field of behavioural ecotoxicology toward increased environmental realism, ecological complexity, and mechanistic understanding. This review identifies research areas in ecology that to date have been largely overlooked within behavioural ecotoxicology but which promise to yield valuable insights, including within- and among-individual variation, social networks and collective behaviour, and multistressor interactions. Further, the review features methodological and technological innovations that enable the collection of data on pollutant-induced behavioural changes at an unprecedented resolution and scale in the laboratory and the field. In an era of rapid environmental change, there is an urgent need to advance our understanding of the real-world impacts of chemical pollution on wildlife behaviour. This review therefore provides a roadmap of the major outstanding questions in behavioural ecotoxicology and highlights the need for increased cross-talk with other disciplines in order to find the answers.

#### **2.10.P Field Studies, Monitoring and Effects Research in Wildlife**

##### **2.10.P-We084 Are Metal Levels in Female Sea Turtles Influencing Their Eggs?**

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Marine turtles are global migrators with a complex life cycle that involves several ecosystems. Due to their long lifespan, as well as their feeding habits, these reptiles tend to accumulate persistent pollutants in their tissues, such as metals and metalloids. These pollutants are widely distributed and are known to have harmful effects in a myriad of organisms. Sea turtles are known to accumulate these pollutants, but few studies have been made to address how the effects of metal pollution in reproductive female individuals may influence their overall health and especially the quality of their eggs to infer on health of future sea turtles'



generations. São Tomé Island hosts important nesting and feeding grounds for four of the seven existing species of sea turtles, including the green (*Chelonia mydas*) and the hawksbill (*Eretmochelys imbricata*) sea turtles. The main goal of this study was to explore if the contamination by different metals and metalloids in the blood of nesting female green and hawksbill sea turtles could be associated with morphological characteristics and composition of their eggs and respective compartments (e.g., total fat, weight from the different compartments, shell thickness, and egg dimensions). In sea turtle eggs, the yolk represents most of the egg mass, and it is highly rich in lipids. Additionally, yolk fatty acids are the major source of energy for embryo development and for the posthatching activity, as both metabolic and signalling mediators. As metals are known to exert a strong negative influence on lipids and fatty acids, this study also aimed to evaluate if the energetic reserves quality for the embryo development (fatty acids in the yolk) in these two species would be affected by the contamination status of their mothers. Results indicate that the screened contaminants significantly affected the egg diameter, shell and membrane weight, shell thickness and lipid reserves. Regarding the fatty acids from the egg yolk, the ones linked to the polar fraction (mostly associated to lipoproteins) seemed to be more influenced by the contaminant levels of the females, where significant correlations were found particularly with fatty acids with higher unsaturation degrees, such as n3 and n6, thus suggesting oxidative events in yolk fat membranes and protein mobilization. Overall, this study showed that how these contaminants present in the blood of female turtles may be affecting their reproductive output by altering egg quality.

#### **2.10.P-We085 Biomonitoring of Metals in Blood and Feathers of Cinereous Vultures (*Aegypius monachus*) Nestlings from do Tejo Internacional Natural Park (Portugal), 2018-2021.**

**Antonio Juan Garcia-Fernandez<sup>1</sup>, Pedro Jiménez<sup>2</sup>, Alfonso Godino Ruiz<sup>3</sup>, Alicia Montano<sup>2,4</sup> and Isabel Navas<sup>5</sup>**, (1)Toxicology and Risk Assessment Research Group, Faculty of Veterinary, University of Murcia, Spain, (2)Health Sciences, University of Murcia, Spain, (3)Hawk Mountain Sanctuary, Portugal, (4)Consejería de Agua, Agricultura y Medio Ambiente de la Región de Murcia, Spain, (5)Health Sciences-Faculty of Veterinary-IMIB, University of Murcia, Spain

The Parque Natural do Tejo International (Tejo N.P.) encompasses a portion of the municipalities of Castelo Branco and Idanha-a-Nova (Portugal), with around 154 breeding bird species, including cinereous vulture (*Aegypius monachus*). To our knowledge, this should be the first study about the presence of several metals in both blood and feather samples, from nestlings of this species. It has been suggested that one the main cause of death in Cinereous vultures could be lead poisoning by consuming of carcasses left by hunters using lead ammunition. Cinereous vulture is considered an endangered species in Portugal, and it is well known that abnormal exposure to some metals can affect species at the population level. The aim of this study is biomonitoring metals in blood and feathers of 34 nestlings of Cinereous vulture from Tejo N.P. (Portugal), to estimate the type of exposure and, if possible, potential effects by Pb and other metals of concern (Cd, Hg, As, Zn, Cu). Samples were collected during 4 breeding seasons: 2018 (n =7), 2019 (6), 2020 (10), and 2021 (11). Analyses were done by ICP-MS after wet digestion in quartz tubes. CRM TORT-2 was used for quality assurance. Median concentrations (ng/g) detected were Zn(115,764) > Cu(5,966) > Pb(785) > Hg(269) > As(255) > Cd(14.4) in feathers, and Zn(5,106) > Cu(404) > Pb(119) > As(6.70) > Hg(2.30) > Cd(0.30) in blood. Zn and Cu are essential trace elements, whilst the rest of elements have not known biological activity. Median concentrations in feathers were significantly higher than in blood, with the following feather/blood rates: Hg(117) > Cd(48) > As(38) > Zn(22) > Cu(15) > Pb(7). Twelve vultures had blood Pb levels below 8.00 µg/dL (background exposure level), 14 were below 20.00 µg/dL (analytical biomonitoring), 7 vultures were below 40 µg/dL (subclinical biomonitoring), and only one vulture had 45.34 µg/dL (preventive intervention). In other words, 24% of the population studied had blood lead concentrations of concern. Concentrations detected in this study will be contrasted with the scarce available published data on this species. In conclusion, although more information about metal exposure in Cinereous vultures is necessary, these results indicate that the exposure to lead should be periodically monitored and studies of biomarkers should be carried out to observe potential effects metal-induced.

#### **2.10.P-We086 Estimation of the Daily Incidence of Lead Ammunition Ingestion in Griffon Vulture with Regurgitated Pellets**

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The birds of prey can be exposed to high lead (Pb) doses by ingesting shotgun pellets or bullet fragments that are embedded in the tissues of the prey they consume. Although birds of prey, including scavengers, can eliminate Pb ammunition by regurgitating it into pellets, the extremely acid gastric fluid of these species favours its partial dissolution and absorption during the digestion process. The objective of this study was to quantify the frequency of Pb ammunition ingestion in griffon vultures (*Gyps fulvus*) to establish whether the prevalence and concentrations observed in vultures in other studies in Spain can be fully explained by the ingestion of Pb ammunition. The study also allowed us to assess the spatiotemporal variations of ammunition ingestion and the relationship with game meat consumption. Fresh pellets (n = 622) were collected throughout all seasons of the year between 2020-2022 at roosting sites in Spain. Pellets were X-rayed and those with the most radiopaque particles were examined and the metal particles retrieved for the identification of element composition by ICP-MS. The main prey present in each pellet was identified by colour and microscopic characteristics of hairs. The X-ray examination revealed the presence of radiopaque particles of Pb (8.5% of pellets), Cu (1.1%), Fe (0.3%), and Bi (0.3%). In summary, 9.6% of pellets contained ammunition fragments, that is, Pb and/or Cu. Prevalence of ammunition ingestion were significantly higher in the periods with large game hunting (11.7%, n = 385) than in the rest of the year (6.3%, n =237, p = 0.011). The studied pellets contained game animals (33.0%), livestock (33.6%), or a mixture of both (33.4%), and the presence of ammunition particles was higher in pellets with game animal remains (17.6%) than in those with livestock remains (3.3%, p = 0.001). Assuming an average pellet regurgitation time of 3 days and a time for elevated (>20 g/dL) blood Pb concentration after ammunition ingestion of 14 days, we may estimate a similar daily

incidence (3-4%) from the prevalence obtained from blood Pb analysis in live free-ranging vultures (44.5%) and from noninvasive monitoring of regurgitated pellets (9.6%). The high rate of ammunition ingestion associated with the consumption of game animals may well explain the high prevalence of blood Pb levels >20 µg/dL according to the similar incidence estimated from blood analysis and noninvasive pellet monitoring.

#### **2.10.P-We087 Fecal Calcium Levels of Bird Nestlings as an Indicator of Species-Specific Sensitivity to Metal Pollution**

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Sensitivity of bird species to environmental metal pollution varies but there is currently no general framework to predict species-specific sensitivity. Such information would be valuable from the conservation point-of-view. Calcium (Ca) has antagonistic effects on metal toxicity and studies with some common model species suggest that low dietary and circulating calcium (Ca) levels indicate higher sensitivity to harmful effects of toxic metals. Here we test the generality of this idea with a larger set of bird-species. We collected fecal samples from nestlings of 66 bird species to estimate levels of their dietary Ca and five other macroelement (Potassium K, Magnesium Mg, Sodium Na, Phosphorus P, Sulphur S) by using fecal concentrations as a proxy measure for diet. We found marked interspecific differences in fecal Ca concentration, which correlated positively with Mg and negatively with Na, P and S levels. Lowest Ca concentrations were found in insectivorous species and especially aerial foragers, such as swifts (Apodidae) and swallows (Hirundinidae) indicating potentially high sensitivity to toxic metals. Instead, ground foraging species like starlings (Sturnidae), sparrows (Passeridae), cranes (Gruidae) and larks (Alaudidae) showed relatively high Ca levels. Independent on phylogeny, insectivorous diet and aerial foraging seem to indicate low Ca turnover and potential heavy metal sensitivity. Our results, together with information published on fecal Ca levels and toxic metal impacts, suggest that dietary Ca levels are a promising new tool to evaluate potential metal-sensitivity of bird nestlings, and we encourage collecting such information on wider range of bird species.

#### **2.10.P-We088 Exploring Environmental Chemical Exposure in Seabird Chicks at Two European Breeding Sites Using a Nontargeted Analytical Approach**

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Chemical pollution of anthropogenic origin affects wildlife in marine habitats across the world. Many environmental chemicals (ECs) can accumulate through the food chain, and long-lived organisms and top predators such as seabirds can suffer particularly severe effects. Seabirds are also threatened by other stressors (i.e., climate change, disease, and shifts in prey abundance). Such pressures are resulting in notable declines in the UK as well as in other European regions. Understanding the role that ECs might play in these biodiversity losses is therefore critical.

While there is much interest in understanding the relationships between ECs and effects, most studies focus on specific groups of chemicals (e.g., metals or organochlorines) using targeted analytical approaches. Most of the research also tends to focus on adult birds – but EC exposure can be particularly harmful in the early stages of life when many important developmental processes are taking place. Therefore, it is important to better understand the specific effects of ECs during pre- and early postnatal life, which can have an impact up to population level.

The aim of this project was to establish comprehensive EC profiles in seabirds breeding at two different European sites: a colony within a national nature reserve in Scotland and a colony in a busy harbour in Belgium, using a non-targeted analytical approach. By sampling from these different sites, a comparison could be made between a more natural environment and a location under strong anthropogenic influence. Work focused on early postnatal exposure in Arctic terns and common terns and possible effects on chick development and health. Both species are long-lived seabirds feeding at a higher trophic level. Samples were screened for different ECs using two-dimensional gas chromatography-time-of-flight mass spectrometry and thus a broad overview of exposure was established.

A better understanding of the impacts of ECs on seabirds will help improve their conservation, enable mitigation of negative impacts and help predict risks to other species foraging in the same habitats. In addition, previously overlooked ECs could be detected with the help of a nontargeted approach.

#### **2.10.P-We089 Exploring VKOR Gene Expression as a Biomarker to Assess Anticoagulant Rodenticide Exposure in Eagle Owl (*Bubo bubo*) Nestlings**

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Anticoagulant rodenticides (ARs) are toxic compounds widely used against rodents, especially in agricultural and urban areas. Owing to their high toxicity and bioaccumulation capacity, they pose a serious risk to exposed predators, leading to secondary

poisoning. However, the factors determining the level of exposure in nontarget wildlife have been poorly investigated. The vitamin K 2,3-epoxide reductase (VKOR) represents the molecular target of ARs and plays an essential role in the vitamin K cycle and activation of clotting factors. Its isoenzymes vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1) and VKORC1-like1 (VKORC1L1) catalyze the same reaction but show a different expression ratio according to tissue type and species. Previous studies on VKOR activity in avian species highlight that owls are particularly sensitive to ARs. The Eurasian eagle owl is considered a suitable sentinel species for biomonitoring contaminants in southeastern Spain, where this species is abundant and likely to be exposed to ARs. As the VKOR enzymes are inhibited by ARs, we hypothesized that VKOR gene expression would increase as a compensatory response. In such case, this measure would be a suitable biomarker of exposure and effect to ARs. The aim of the present study is to analyse VKORC1 and VKORC1L1 gene expression in different scenarios with possible sources of ARs contamination. For this purpose, 72 samples belonging to Eagle owl (*Bubo bubo*) chicks from 26 nests were collected in the Region of Murcia (Spain), close to potential sources of ARs pollution (< 2 km). These included fruit tree crops, intensive livestock farms, landfill sites, and human settlements. Some of the sampled nests were located in natural areas (control sites). The results show that the total expression (sum of the expression of VKORC1 and VKORC1L1) is significantly higher in owlets from nests located near residential areas than in control areas ( $p = 0.012$ ). Though not significant, a similar trend is found in owlets from nests in the close vicinity of fruit orchards. These findings suggest that Eagle owl populations living near urban areas are more exposed to ARs, which increases the risk of suffering its harmful effects. Furthermore, this study indicates that VKOR mRNA expression may be a promising biomarker of ARs exposure for Eagle owl as well as for other nontarget species.

**2.10.P-We090 Exposure Assessment of Anticoagulant Rodenticides in Liver of Red Foxes (*Vulpes vulpes*) in Slovenia**  
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Due to their mass use, anticoagulant rodenticides (ARs) pose a significant environmental problem with toxic effects on nontarget wildlife. For this reason, residues of the first generation ARs chlorophacinone, coumatetralyl, and warfarin and of the second generation ARs brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen were monitored in red foxes (*Vulpes vulpes*) in Slovenia to assess their bioaccumulation and the occurrence of secondary poisoning.

In 2019–2022, 148 liver tissue samples were collected from legal and regulated hunting of adult red foxes from all geographical regions of Slovenia. The samples were extracted with methanol/water (2:1, v/v), cleaned-up using a solid supported liquid-liquid extraction, and measured by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS) with reporting limits in the range of 0.5–5.0 ng/g. Residues of at least one rodenticide were detected in 77.7% of the analyzed livers. The second generation ARs bromadiolone, brodifacoum, and difenacoum were most frequently found in 75.0, 51.4, and 18.9% of the samples, respectively. Concentrations of pooled ARs ranged from 1.5 to 2866.5 ng/g with a mean and median value of 601.4 and 350.2 ng/g, respectively. We determined bromadiolone at concentrations of  $\geq 800$  ng/g in 16 of 148 fox livers (10.8%) and two of them (1.4%) contained residues  $>2000$  ng/g. The distribution of bromadiolone and brodifacoum showed no significant differences across sex and body mass categories of the animals included in the study. First generation ARs were found in only 9.5% of the samples, the residues were with one exception (coumatetralyl with 55 ng/g) below 10 ng/g. Chlorophacinone and difethialone were not detected. The results of the study demonstrate a serious toxicological risk for red foxes in Slovenia.

To the best of our knowledge, this is the first study to assess the exposure of nontarget animals in the Western Balkan countries to ARs. The findings highlight a potential environmental problem related to persistence, bioaccumulation, and toxicity of ARs in this region and will contribute to the knowledge on the protection of European ecosystems.

**2.10.P-We091 Greater Predisposition to Anticoagulant Rodenticide Exposure in Red Foxes (*Vulpes vulpes*) Affected by Canine Distemper Disease**

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Anticoagulant rodenticides (ARs) exposure in wild carnivores is a global concern due to their continuous and widespread use worldwide. We studied the prevalence of ARs in liver samples of 25 Red foxes (*Vulpes vulpes*), 3 European badgers (*Meles meles*), and 2 Genets (*Genneta genneta*) from Alicante (Spanish Levante region) obtained in 2021 and 2022. All samples came from Santa Faz Wildlife Rehabilitation Center (Alicante, Spain) where the necropsies were performed. The most frequent causes of death in wild carnivores in this region are trauma and poisoning by pesticides. However, during this period of study there were several foxes with suspected infectious clinical disease. All commercially available AR active ingredients in Europe were included in this study. So, both first and second generation ARs were analysed by HPLC/MS/TOF in liver samples after a QuEChERS extraction procedure. Apart from the cause of death, the influence of other variables such as age, sex and location were also assessed in ARs liver concentrations. Potential health risk for individuals and populations in the study area have been studied. In relation with this, higher ARs concentrations have been detected in a group of red foxes affected by distemper virus compared to another group of healthy red foxes, mostly killed by trauma. Therefore, the present research aims to explain a possible association between the fact of suffering from an infectious disease and the increase in ARs concentrations of affected animals. Furthermore, we expect to confirm the hypothesis that Red fox (*Vulpes vulpes*) is considered a good sentinel species for the risk of exposure to ARs. All the samples analysed contained ARs and the most detected compounds were difenacoum and

bromadiolone (100% of the samples), brodifacoum (93.33%), and flocoumafen (86.67%). Additionally, 53.33% of the animals had at least one second generation anticoagulant rodenticide (SGAR) above the threshold value reported as triggering adverse health effects (200 ng/g). Regarding this, the level of risk in wild mammals in Levante region could be classified as high and worrying. Moreover, we suggest that individuals and populations with weakened health due to other diseases (for example, infectious) could be more prone to high exposure to anticoagulant rodenticides and, very probably, would be more sensitive to suffering serious effects at lower doses of ARs.

#### **2.10.P-We092 High Exposure to Anticoagulant Rodenticides as Cause of Poisoning and Mortality in Bonelli's Eagles from Southeastern Spain**

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Anticoagulant rodenticides (ARs) comprise one of the most toxic groups of compounds currently used worldwide for rodent pest control. Some of them are highly persistent and bioaccumulative. Toxic baits are often, directly or indirectly, ingested by nontarget animals, resulting in secondary poisoning, and affecting frequently apex predators.

Their presence in many species of raptors is quite common, especially in scavenger species serving as sentinel for the presence of these substances in the environment. However, there is little data on the presence of AR in Bonelli's eagle, one of the most endangered eagle species in Spain and with a negative population trend in Europe. This medium-sized eagle feeds entirely on live species that it captures before feeding.

In this study, 17 carcasses of Bonelli's eagles from Valencian Community (Eastern Spain) were necropsied. Both first and second generation ARs were extracted from the livers using a modified QuEChERS technique.

Five first generation ARs (Coumatetralyl, Diphacinone, Warfarin, Coumatetralyl, Chlorophacinone) and five second generation ARs (Bromadiolone, Brodifacoum, Difenacoum, Difethialone, Flocoumafen) were analyzed. Analysis of ARs by HPLC-MS revealed that all eagles studied were exposed to at least 6 ARs, out of a total of 10 ARs analyzed, with 8 being the highest number of ARs detected in a sample. Second generation ARs were the most prevalent. 35% of the eagles showed a concentration higher than 200 ng/g of ARs, being compatible with AR poisoning. Bromadiolone and Brodifacoum were the most predominant ARs and they were also the compounds with the highest concentrations, in 94% of the cases.

In conclusion, ARs are widespread in the Spanish Levant, affecting nontarget species and reaching higher trophic levels. The high presence of these compounds in Bonelli's eagles could be a new cause of mortality for this species or could explain other causes of death like the high mortality in power lines and should be taken in account for their conservation. At the same time, the presence of these compounds in the environment represents risks to public health, as the most frequent species in the diet of Bonelli's eagle (rabbits and partridges) are hunted and consumed by humans.

#### **2.10.P-We093 Influence of Seasonality on the Temporal Trend Assessment of Wildlife Exposure to Second-Generation Anticoagulant Rodenticides: A Case Study for UK Common Buzzards (*Buteo buteo*) from 2001 to 2019**

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Avian predators are widely exposed to second-generation anticoagulant rodenticides (SGARs) by feeding on contaminated prey. Some studies have demonstrated a strong seasonal variation in the exposure of raptors to SGARs, likely linked to seasonal variation in their diet. However, many previous biomonitoring studies have not included seasonality in their models, which might lead to misleading conclusions on the risk assessment of SGARs. In this study, we modelled temporal trends of SGAR residues in Common Buzzards (*Buteo buteo*) with and without seasonal fluctuation to investigate how the inclusion of seasonality within the model modifies conclusions on temporal exposure trends of wildlife to SGARs.

We used 72 buzzards found dead in the wild in the UK between 2001 and 2019. For each carcass, the month of the collection was recorded, and tissue samples were collected after a postmortem examination. Liver concentrations of five SGARs (bromadiolone, difenacoum, brodifacoum, flocoumafen, and difethialone) were measured. The detection/nondetection in buzzards of each SGAR and summed SGARs ( $\Sigma$ SGARs) over time was analysed by logistic models with and without harmonic terms (which explain seasonal fluctuation) and statistically compared. We applied the same approach, using linear models, for liver concentrations of each SGAR and  $\Sigma$ SGARs in the samples excluding non-detect SGAR ones.

Our initial results suggested that the detection of bromadiolone, difenacoum, and  $\Sigma$ SGARs was better explained by logistic models including seasonality, although models without seasonality showed similar temporal trends in detection over the period. In contrast, temporal trends of SGAR liver concentrations were better explained by linear models without seasonality, except for difenacoum whose liver concentrations were better explained by the inclusion of seasonality.

Overall, seasonality influenced the detection/non-detection of SGARs in buzzards but was not important for modelling hepatic concentrations of most SGARs over time when nondetect SGAR samples were excluded from the analysis. Our results indicate that excluding seasonality as a variable might not necessarily lead to misleading conclusions on biomonitoring. However, opportunistic avian predator biomonitoring protocols do not systematically collect samples in the same season or do not systematically exclude non-detect SGAR samples. Therefore, seasonal variation in the detection of SGARs may cause a bias in risk assessment.

## 2.10.P-We094 Targeted Sampling for Brown Rat Resistances Identification and Incidence in Urban Ecosystems: The City of Madrid

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Brown rats (*Rattus norvegicus*) are commensal rodent species found in towns and one of the most frequent urban pest. Their control is crucial for authorities in order to avoid health and economic issues. Nowadays, the only effective control method available is the use of anticoagulant rodenticides (ARs) that inhibit the enzyme vitamin K 2,3-epoxide reductase (VKORC1), responsible for reducing vitamin K. The inhibition of VKORC1 prevents the activation of the coagulation factors resulting in animal death by internal bleeding. Resistance to AR is associated to *vkorc1* mutations and have been found through random sampling of Pest Control Operators (PCOs) worldwide. In the present study, we aim to introduce targeted sampling procedure as a useful tool for pest controllers' authorities and sanitation companies in the urban environment. Sampling was designed with the aim of inferring the results to the general population of rats in Madrid Central Almond with an area of 4.8 km<sup>2</sup>. The districts were divided into grids of 1000 x 1000 m to ensure the collection of a sufficient number of samples that encompass the largest number of rat colonies. Design also included the selection of sites of at least one segment or section of the sewer system practicable for at least 100 m long. In each collector, six points of sampling of faecal deposits were spaced at least 20 meters to guarantee individuality. Geospatial analysis was done to relate population density, rat warnings, bromadiolone use, and trash production with *vkorc1* mutation frequency. A total of 126 stool samples were received from the Department of Vector Control of the City Council of Madrid from 16 neighbourhoods of seven districts. Mutation frequency varies among neighbourhoods and is dispersed along the main central road. The S149I mutation is most frequent in the south and the E155K in the north while double mutant is found in the central district. Interestingly, increase in mutation frequency shows a tendency towards high trash production. The mutation frequency map is an instant moment of what has been done in the past and indicates the areas to change the use of AR and rodent environmental food supply. Therefore, the design of an appropriate sampling would improve control management following resistance evolution over time and space by monitoring the genetics of *vkorc1* in the rodent populations.

## 2.10.P-We095 Pesticides and Biocides in the Danish Population of European Hedgehogs (*Erinaceus europaeus*)

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European hedgehogs (*Erinaceus europaeus*) inhabit most of Denmark. Long-term reduction of suitable habitats causes hedgehogs to increasingly inhabit areas with human occupancy, e.g., gardens, parks, and other recreation spaces, with the potential for pesticide and biocide exposure. In addition, hedgehogs inhabiting rural areas may come into contact with pesticides applied during agricultural practices. The exposure of hedgehogs to potentially toxic chemicals could be a contributing factor to the decline of this species.

Our research studied the occurrence of various pesticides and biocides in European hedgehogs from Denmark and investigated potential risks of exposure by testing for links to age, sex, location, degree of inbreeding, cause of death, and habitat type of the animals.

We used liver samples obtained from dead hedgehogs collected in Denmark in 2016 as part of a citizen science component of The Danish Hedgehog Project. We analysed a selection of commonly used rodenticides (N = 6), insecticides (N = 5) and herbicides (N = 8) in 115 individuals, using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Furthermore, livers from 17 individuals were selected for non-targeted screening with LC-QToF-MS and GC-Orbitrap-MS.

The targeted analyses showed detection frequencies (DFs) between zero and up to 79% for bromadiolone, with a median value of 12% and an interquartile range (IQR) of 3%-30% across all compounds. Besides bromadiolone, the compounds detected most frequently included the rodenticides coumatetralyl (49%) and brodifacoum (32%) as well as the insecticide imidacloprid (35%). Concentrations varied between nondetected to >2 µg/g. Our preliminary data analysis indicated that the presence of pesticides and biocides did not appear to be affected by sex, age, degree of inbreeding, or habitat type. The DFs were found to differ significantly between localities for difenacoum (Jutland (47%) versus Zealand (22%)), difethialone (Zealand (38%) versus Jutland (16%)), and imidacloprid (Zealand (46%) versus Jutland (23%)).

The GC- and LC-based nontargeted analyses revealed 35 and 38 potential pollutants, respectively. Among those were legacy persistent organic pollutants (POPs), such as p,p'-dichlorodiphenyldichloroethane (DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs). In addition, several pharmaceuticals were identified.

We conclude that European hedgehogs are exposed to a diverse range of anthropogenic compounds in Denmark.

## 2.10.P-We096 Relationship between Pesticides and Emergent Diseases in Wildlife Inhabiting Agrosystems: Glyphosate and Myxomatosis in the Iberian Hare

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Pesticide consumption has increased exponentially in the last decades around the world, largely due to its use in agriculture. In this sense, wildlife species inhabiting agrosystems are potentially continuously exposed to a large amount of pesticides. Although pesticides mode of action on target species is often known, their secondary or sublethal effects on wildlife in general are not known but are expected to modulate population dynamics. Another important factor involved in modulating wildlife populations is infectious diseases, especially emerging diseases, which often appear as outbreaks with high mortality rates. In this context, wild lagomorphs, strongly associated with agrosystems, appear as excellent animal models and sentinels of exposure to pesticides and pathogens, due to their ecological relevance as keystone species and their socioeconomic value as game animals. Therefore, this work aimed to study the exposure to pesticides and emerging pathogens on wildlife, using the Iberian hare (*Lepus granatensis*) as a model species. This species is since 2018 hit by a highly virulent emerging disease, myxomatosis. For this purpose, glyphosate residues were analysed together with the seroprevalence of myxomatosis in hunted animals from pesticides-treated and pesticide-free areas during the 2021/22 hunting season in the southwestern Spain. Glyphosate residues were analysed in samples of gastric contents based on a method previously developed for our research team with some modifications for high performance liquid chromatography coupled to triple quadrupole with linear ion trap (Q-TRAP). For myxomatosis, we considered both animals with compatible lesions during necropsy, as well as with antibodies for this virus in serum samples by ELISA techniques. The results are expected to represent a significant scientific breakthrough due to the widespread use of pesticides worldwide, the population decline observed in many wild species associated with agrosystems, and the controversy over chronic toxic effects on nontarget organisms, including their synergistic effects with emerging infectious diseases.

#### **2.10.P-We097 Relationship between the Effects of Pesticides and Disease in Iberian Hare Female Reproduction**

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In recent decades, species associated to agrosystems have declined significantly worldwide. Numerous factors have been identified as possible causes of these declines, including environmental pollution (largely due to the increase in the use of pesticides in the 20th century). Another important factor involved in modulating wildlife populations is infectious diseases, especially emerging diseases, which often appear as outbreaks with high mortality rates. While the effects of some wildlife infectious diseases on population dynamics are well established, the combined effects that pesticides and pathogens can produce remain largely unknown. Therefore, this study analyses for the first time the independent effects of exposure to pesticides, as well as their combined effects with emerging pathogens, on the population dynamics of wildlife inhabiting agrosystems. More specifically, we have analysed, by histological study, the number of different ovarian follicles in females hunted Iberian hare (*Lepus granatensis*) in pesticide-treated and pesticide-free areas with and without symptoms compatibles with myxomatosis or myxomatosis antibodies during the 2021/22 hunting season in the southwestern Spain. Species affected by this emerging disease since 2018. Preliminary results showed lower number of corpora lutea in individuals from treated areas than pesticides-free areas. In addition, individuals with myxomatosis tended to have lower number of primary follicles, tertiary follicles, atretic follicles and corpora lutea than animals without myxomatosis, although the differences were not significant. Overall, the results suggest that in intensive agricultural areas pesticide exposure could have reproductive effects by decreasing the number of ovarian follicles, effect that can act synergistically with the effect of diseases. We discuss the implications of these results from the perspective of the conservation of this species.

#### **2.10.P-We098 Relationship between the Effects of Pesticides and Disease in Iberian Hare Male Reproduction**

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Pesticides and emerging diseases have been identified among the five most important threats to biodiversity globally. Concerning pesticides, there are important shortcomings in the knowledge of secondary or sublethal effects on wildlife and therefore, its potential impact at population level. While the effects of some wildlife infectious diseases on population dynamics are well established, the combined effects that pesticides and pathogens can produce remain largely unknown. In this context, this study analyses the independent effects of exposure to pesticides, as well as their combined effects with emerging pathogens, on the population dynamics of wildlife, using the Iberian hare (*Lepus granatensis*) as a model species. In 2018, the first outbreak of myxomatosis, an infectious disease caused by myxoma virus, was described in Iberian hares in southern Spain. Since then, myxomatosis has threatened hare populations throughout the Iberian Peninsula. Infected animals show severe vascular changes,

including at the testicular level. Testicles were then collected from male hares hunted in pesticide-treated and pesticide-free areas, with and without symptoms compatibles with myxomatosis or myxomatosis antibodies during the 2021/22 hunting season in the southwestern Spain. Testes and epididymis, obtained on the day of capture, were weighted and conserved at 5°C until processed the following day (12h). Total cauda epididymal spermatozoa were counted, and the percentage of morpho-anomalies determined by eosin/nigrosine stain. A battery of sperm parameters was determined by a computer assisted semen analysis, and by flow cytometry. Preliminary results showed that both factors (pesticides exposure and myxomatosis) appear to affect the parameters analysed, and therefore be key modulating factors in the population dynamics of this species.

## **2.10.P-We099 Residues of Glyphosate, AMPA, and Glufosinate in Soils, Earthworms, and Wild Small Mammals in Arable Landscapes: A New Case of “Emerging Organic Contaminants”?**

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Since their registration in the 70's the use of glyphosate-based broad-spectrum herbicides constantly increased, making glyphosate (GLY) one of the most widely applied herbicide worldwide nowadays. Accumulating evidences show that GLY and its metabolite AMPA may be more persistent in the environment and more toxic to biota than expected based on risk assessment in marketing authorization. Recent debates about GLY use mainly focused on human health issues while little is known about risks to wildlife. Glufosinate (GLUF) could be an alternative compound to the use of GLY-based herbicides, but its field ecotoxicology has rarely been studied.

We investigated residues of GLY, AMPA, and GLUF in soils ( $n = 120$  locations), in earthworms ( $n = 120$ ), and wild small mammals ( $n = 63$  rodents and shrews) sampled in treated (e.g., cereal fields under conventional farming) and nontreated habitats (e.g., hedgerows and cereals under organic farming) over arable landscapes in France. GLY, AMPA, and GLUF were detected in 88%, 58%, and 35% of the soil samples, in 74%, 38%, and 12% of the earthworm samples, and in 64%, 51%, and 44% of small mammal hair samples, respectively. The detection of GLY, AMPA, and GLUF was higher in herbivorous *Microtus* and granivorous *Myodes* voles than in insectivorous *Crociodura* shrews and omnivorous *Apodemus* wild mice. The compounds were found in samples from both conventional and organic cereal fields without significant differences according to the cropping system, as well as in hedgerows and grasslands. GLY and AMPA were more frequently detected and at higher concentrations in soils from cereal fields and hedgerows and in earthworms from cereal fields. The concentrations of GLY were higher in small mammals captured in hedgerows than in cereal fields.

The bioaccumulation of GLY and AMPA was evidenced in earthworms and found greater than expected based on physicochemical features of the compounds. Soil concentrations were lower than Predicted Environmental Concentrations but the maximum GLY concentrations reached values only 2 to 3 times lower than doses showed to have negative effects on earthworms (survival, avoidance).

Showing a ubiquitous soil contamination and chronic exposure of fauna to GLY, AMPA, and GLUF, our findings raise issues about the occurrence of the compounds at landscape scale even in non-treated and semi-natural areas considered as refuges. This study also highlights the potential for transfer of GLY, AMPA and GLUF in terrestrial food webs.

## **2.10.P-We100 Rice and Pesticides: How Should we Assess the Environmental Risk?**

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Rice is an essential crop for humans and, at the same time, rice fields are essential habitats for waterbirds. Therefore, the management of rice fields should incorporate bird biodiversity while securing rice production. We propose a field methodology that will generate useful data for better management of rice fields. In the first stage of the project, we characterized multiple rice fields according to environmental features and agronomic factors, and we collected information on the crop yield and bird populations.

The study was performed in the Ebro Delta, an important rice-producing area in Spain and an area of international importance for waterbird conservation. We conducted surveys among farmers to collect information on PPP usage, sowing method (dry vs. wet), soil characteristics, rice variety, field headland management, and yield. For the selected fields we also characterized the surrounding landscape and field headlands. Additionally, bird point counts of 5 minutes duration were performed in each rice field in 3 sampling periods.

We collected information on 76 conventional and 20 organic fields. The average vegetation coverage and width of the field headlands were calculated for each field. Coverage and width were significantly higher ( $p \leq 0.001$ ) in organic fields than in conventional fields. In conventional fields, a total of 21 different active substances were used: 12 herbicides, 6 fungicides, and 3 insecticides. Conventional rice fields were grouped into four categories according to the number of compounds they used (i.e., 0-3; 4; 5-7; 8-12). Glyphosate was used in the field headland of 95% of the conventional fields and 25% used also MCPA.



Regarding treated seeds, 54.7% of the conventional fields used insecticide-treated seeds, and 18.7% used also a fungicide. The number of bird species detected per field and number of individuals per field were significantly higher in organic fields than in conventional fields (both  $p \leq 0.001$ ), which suggests that birds are responding to changes in the rice field (e.g., habitat diversity).

Collected information will be used to build and test risk exposure scenarios. This preliminary analysis is essential to adequately define experimental units for the next stage of the project (i.e., measurement of pesticide residues in environmental samples and analysing the effects of pesticide exposure on birds and invertebrates).

## 2.11 Filling Gaps for Micro- and Nanoplastic Effects and Risk in Multiple Stressed Aquatic Environments

### 2.11.T-01 Effects of Nano-, Microplastics, and Temperature on Crustaceans

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Effects of micro- and nanoplastic particle exposures on aquatic wildlife are still being explored, while there are knowledge gaps about yet untested interactions with temperature. Especially, the effects of fibers are not well understood. The goal of our studies was to evaluate whether particle sizes, polymer types, and sublethal concentrations impacted organismal responses in the model species *Hyalella azteca* and *Americamysis bahia*, at three temperatures (21–28 °C). Across two studies we exposed *H. azteca* for 96 hours to 0.43 mg/l of fluorescent polystyrene beads of nano- (500 nm) and micro- (1000 nm) size and *A. bahia* for 7 days to cotton, nylon, PES, and HEMP fibers (ca. 4 p/ml and in average 200 µm long), and to PLA and LDPE, as well as to their leachates (1–20 µm, up to 500 p/ml). Following exposure at three temperatures, we evaluated particle uptake, oxidative stress, and multiple behavioral endpoints.

Initial results show high variability in uptake and no translocation of particles or fibers from the gut into adjacent tissue. Mortality was significantly higher following exposure to 500 nm polystyrene beads at the highest temperature of 27 °C than in respective controls without particles. Furthermore, with rising temperature, the uptake of 1000 nm polystyrene particles tended to increase. Fiber ingestion resulted in elevated oxidative stress corresponding with increasing temperature. Whilst no impact on total mobility was observed, videotracking analyses integrating multiple behavioral endpoints allowed for detection of more sublethal effects. Together, our results demonstrate greater effects of particle uptake at higher temperatures. Climate change predicted increases in surface water temperatures may pose greater risk to aquatic organisms with further increases of nano- and microparticles in aquatic ecosystems.

### 2.11.T-02 How do Microplastics Exacerbate Virus-Related Mortality in a Commercially Valuable Salmonid Species?

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Microplastics are an environmentally ubiquitous threat, coexisting with a variety of natural and anthropogenic stressors. In the aquatic environment, for example, pathogens commonly impact multiple trophic levels and human resources. We evaluated the extent to which microplastics mediate the virulence of an important marine disease (Infectious Hematopoietic Necrosis virus, IHNV) in rainbow trout (*O. mykiss*). Populations of *O. mykiss* are farmed for human consumption across the U.S. North Pacific, Europe, and Japan, where IHNV can be highly destructive. To evaluate the hypothesis that microplastics modulate population mortality or incidence of IHNV, we conducted a controlled experiment in which trout were chronically exposed to varying doses of microparticles (0, 0.1, 1, and 10 mg L<sup>-1</sup>) over an 8-week period, with acute IHNV exposure at four weeks. Microplastics used reflected polymers common in aquaculture and wild-caught fisheries: nylon fibers (~10 x 500 µm) used in nets, and ground expanded polystyrene (fragmented to ~20 µm diameter) used in floats. To evaluate if polymers of natural origin (cellulosic plant matter) induce effects, marsh grass (ground to ~20 µm) was included. Mortality was higher when microparticles and IHNV were co-administered than IHNV exposure alone, increasing significantly for nylon fibers at the high concentration and polystyrene at medium concentration. We observed that fish co-exposed to virus and microplastics (particularly nylon microfibers) had a higher viral load and shed than those exposed to virus alone. Importantly, no significant mortality from the presence of any microparticle exposure in the absence of virus co-exposure was observed, underscoring the importance of microplastics as a co-stressor and not just a singular threat. Further, in a second experiment, fish exposed to microfibers chronically prior to IHNV were more susceptible to disease, while microfiber exposure post viral exposure did not augment mortality. We propose that the increase in relative disease virulence arose from increased susceptibility to infection, which may have also enhanced disease transmission between individuals. This work demonstrates that microplastics are an ecologically significant co-stressor for aquatic biota. This may have implications for human diseases transmitted through seafood, and approximate respiratory stressors (particle and virus) for humans, warranting further multidisciplinary research.

### 2.11.T-03 Multistressor Effects of Chemicals and Microplastics in Fresh-Water Environments

**Katie Reilly**, **Laura-Jayne Ellis**, **Jon Sadler** and **Iseult Lynch**, University of Birmingham, United Kingdom

Microplastics (MP) has been well established as a contaminant of concern, with an increase in scientific and societal interest. The presence of multiple stressors, such as chemical pollutants in combination with MP, poses a complex challenge for ecotoxicology studies to replicate the various pathways of exposures within controlled and simplified testing scenarios to understand the drivers of observed toxicity. *Daphnia magna* are a well-established test organism for chemical testing in freshwater environments.



Standard tests and protocols (for chemical testing) have been established, including the 48-hour acute, and 21-day chronic reproductive, toxicity assays. As a result, there is a wealth of data for chemical effects in *Daphnia* which can be used to establish a baseline response to aquatic pollution stressors, and to rank chemicals based on their toxicity.

*Daphnia* were initially exposed to three chemicals; triclosan (an antimicrobial), Sodium Dodecyl Sulphate (SDS - a surfactant), and diclofenac (a nonsteroidal anti-inflammatory) which allowed an initial dose response to each of the chemicals to be calculated. To determine the effect of environmental factors, *Daphnia magna* were cultured in three different media (salt only, natural borehole water, and artificial river water with added humic acid as a representative natural organic matter (NOM)) to represent different levels of environmental conditions, before being exposed to the three chemicals again, without or with polyethylene MP (1–4 µm) as a second toxicant. Both acute toxicity with dose response curves, and variability in total protein adsorbed onto the MP particle surfaces at the end of the exposures to chemical and medium mixtures, was ascertained.

We found that variability in toxicity depended on both the exposure scenario and the chemical properties of the pollutants. Triclosan and diclofenac appear to be influenced by co-binding to NOM bound onto the MP particles in this study. SDS readily binds to the surface of the particles due to the amphiphilic nature of the chemical and the hydrophobic surface of the MP, and had elevated toxicity within the exposures containing MP particles, demonstrating a change in exposure pathways as a result of co-exposure. It is essential to consider this at the test design stage to take into consideration the environmental factors that could be drivers for chemical and MP mixture toxicity, to increase the realism of the exposure risk calculations.

#### **2.11.T-04 Combined Effect of Salinity and Leachates of Environmental Plastics on the Copepod *Nitokra spinipes***

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Plastic debris have reached approximately a volume of 8 million tons per year in the oceans and it is necessary to understand the effects on the communities that inhabit them. It is essential to consider that plastic litter comprise not only the polymer that makes them up, but also the various additives that are added during their manufacture and the chemicals that might be sorbed to them from the environment, that are often leached because of their persistence and durability. Coastal areas such as estuaries are transitional zones with natural salinity gradient, that can accumulate plastic litter, and because the release of leachates also depend on such environmental conditions, it is therefore important to assess combined stresses to understand the effects on organisms. The main goal of this study was to analyze the ecotoxicological effect of leachates from environmental and weathered plastics, in two brackish water conditions (two salinities), in a representative model species of planktonic organisms, the harpacticoid copepod *Nitokra spinipes*. Three different environmental samples of PVC, PE, and PP collected in coastal areas from Belgium (North Sea) and Argentina (Río de la Plata estuary) were used for the leachates. The plastics were cut into small fragments (2.5 cm) and the leachates (80 g/L) were prepared in brackish water (BW, salinities of 7 and 15) following standardized method (22°C in dark, 7 d, 80 rpm). After 7 days the leachates were filtrated by 1 and 0.2 µm pore size cellulose filters. The toxicity test to assess the mortality in the copepods was carried out for 96 h following the ISO 14669 protocol, comparing the endpoint among the three polymers and using BW as control (0 % leachates), as well as a positive control using dodecanol. The results indicated a 32% of mortality at 24 h caused by leachates (100% concentration) from PVC, being the mortality of 30% at the end of the experiment ( $p < 0.05$ ), at salinity 7. Leachates from PP (2.5% mortality) and PE (7.5% mortality) had no effect on the copepods, compared to the control (5% mortality). The test of ecotoxicological effects of leachates at salinity 15 are currently ongoing. Our results will contribute to assess the combined effect of salinity and leachates of different polymers from environmental stranded plastics, providing information on the complexity of plastics as a diverse group of pollutants with various chemical characteristics, in estuarine ecosystems.

#### **2.11.T-05 Long-Term Effects of Microplastics on a Benthic Community: A Mesocosm Experiment**

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However, little information is available on their long-term effects at higher levels of biological organization. From an ecological point of view benthic organisms play an important role in reworking and irrigating sediments and are important food source for organisms at higher trophic levels. Consequently, effects on benthic fauna will potentially affect not only benthic communities and ecosystems but also the pelagic food web through transfer of MPs from benthic organisms to pelagic predators. However, scientific literature on MPs in sediments is limited resulting in knowledge gaps related to population- and community-level effects of MP. While most experimental studies focus on single polymer exposures, it is very unlikely that organisms would be exposed to only single polymers in environment, but they are rather exposed to a suite of different polymers. To address this data gap, we performed a five-week large-scale mesocosm experiment to address potential population- and community-level effects of MP under environmentally relevant exposure conditions. We used a suite of polymers tested at both environmentally relevant concentration and a 1000-fold higher concentration. To address this data gap, we performed a five-week, large-scale mesocosm experiment to address potential population- and community-level effects of MP exposure to a suite of MPs at an environmentally relevant concentration (680 MPs L<sup>-1</sup>) and a 1000-fold higher ( $6.8 \times 10^5$  MPs L<sup>-1</sup>). We exposed specimen of *Macoma balthica*, *Corophium volutator*, and *Monoporeia affinis* in a community structure representing species diversity and abundance in the area of collection. In parallel, we ran complementary experiment for three weeks with *M. affinis* exposed to

sediment-associated MPs with the MP mixture at environmental and high concentration, eight additional treatments with each of the single polymers at two different concentrations. Endpoints included survival, growth, and ingestion rate. We found no effects of survival, growth, or significant changes in community structure after five weeks of exposure, nor did we observe effects on survival, growth, and ingestion rate in the complementary experiment. These no-effect results are important to consider in the context of MP risk assessment in sediment.

## 2.11.P Filling Gaps for Micro- and Nanoplastic Effects and Risk in Multiple Stressed Aquatic Environments

### 2.11.P-Tu076 Impact of Microplastics on Mussels Under Extreme Climate Conditions

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Microplastics (MPs) is an emerging contaminant that can affect the marine environment from biota to ecosystems. Co-occurring environmental stressors need to be incorporated into the investigation of the toxicity of MPs, as plastics do not act as isolated stressors in aquatic systems. The present study aims to investigate the toxicity of MPs in combination with thermal stress, under the perspective of environmental relevance and a marine heatwave, in marine mussels, a commercially important organism and a marine pollution indicator species.. In particular, the effect of aged MPs on mussel haemocytes will be investigated after the exposure of mussels to a simulated marine heatwave. The MPs will be artificially aged by sonication and the weathering status of the MPs will be evaluated by the determination of the carbonyl index, an indicator of ageing that is based on the oxidation of polymers such as polyethylene or polypropylene. Mussels will be exposed to a simulated heatwave (i.e., increase of water temperature of 5 °C) for a 3-day duration. Then the mussel haemocytes will be extracted and exposed in vitro to a set of MPs treatments and different concentrations from low to high levels including environmentally relevant concentrations. The aim of the exposure is to evaluate whether the heatwave increased the mussel sensitivity to MPs, and if MPs toxicity can be attributed to the aging of MPs (compared to pristine unaged particles). Cell toxicity will be evaluated by cell viability, phagocytosis ability, and evaluation of biomarkers through gene expression. The biomarker analyses will include investigation of the expression of genes involved in the oxidative stress, immune and heatshock response. In a warming climate, environmental stressors such as heatwaves will be increasing in frequency, extent, and magnitude creating the need to understand and predict biological responses to short-term extreme events. The present study will contribute to the evaluation of a realistic scenario of the interaction of MPs in the environment with other relevant stressors.

### 2.11.P-Tu078 Combined Effects of Global Warming and Plastic Leachates from Conventional and Bio-Based Polymers on a Harpacticoid Copepod

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Global warming and plastic pollution are two human-induced environmental stressors of rising concern due to their potential impact on ocean health. To tackle sustainability concerns on the production of conventional petroleum-based polymers, bio-based polymers, i.e., polymers originated from natural feedstocks, are seen as a potential alternative. However, just as conventional polymers, plastic items from bio-based polymers can leach additives and other associated substances into the (marine) environment. To date the ecotoxicological effects of leachates from bio-based polymers are still unclear, but previous reports have demonstrated that leachates from petroleum-based polymers can induce adverse effects in marine invertebrates. Also, in the environment, organisms are subjected to a myriad of environmental stressors, among which global warming, and the combination of stressors is often not considered in environmental risk assessments. To increase our understanding of the combined effects of plastic leachates from either bio-based or petroleum-based polymers and elevated temperature (global warming), in this work we used the case study of self-reinforced polylactic acid (SR-PLA) and self-reinforced polypropylene (SR-PP), at control (22°C) and elevated water temperatures (25°C). To do so, we exposed newly hatched larvae of a harpacticoid copepod *Nitokra spinipes* to plastic leachates (80 g/L) at each temperature. Our preliminary results indicate that after 6 days exposure, lower larval development ratio (%) was found in 60% v/v of SR-PP leachate ( $P < 0.05$ , Dunnett's test) exposure than no leachate controls at 25 °C, but not in SR-PLA leachate exposure ( $P = 0.51$ , Dunnett's test). Larvae at elevated temperature (25°C) had a higher mortality compared to control temperature (22°C) ( $P < 0.05$ , ANOVA). We anticipate that our results will contribute to assessing the impacts of bio-based polymers in multiple stressor environments and the use of more realistic scenarios in environmental risk assessment.

### 2.11.P-Tu079 Disclosing the Effects of Pristine and Weathered Micro- and Nanoplastics Combined with Environmental Contaminants on Fish Intestinal Cells (RTgutGC).

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Microplastic (MPs, 1 µm - 5 mm) and nanoplastics (NPs, <1 µm) are ubiquitous in the environment. Micro- and NPs in nature are exposed to weathering conditions, including photodegradation, that may modify surface charge and reactivity, and release plastic impurities such as trace metals, additives or solvents. Moreover, MPs/NPs ability to act as vectors of legacy pollutants and

modulate the bioavailability, bioaccumulation and toxicity of these contaminants has been highlighted in recent studies. Here, rainbow trout (*Onchorhynchus mykiss*) intestinal cells (RTgutGC) were exposed to polyethylene MPs (pristine and oxidized) and NPs, all UV aged or not. Moreover, to assess the role of MPs/NPs on the bioaccumulation and toxicity of heavy metals and organic pollutants, cells were exposed to MPs/NPs in presence or absence of cadmium and lindane/DDE as a proof of concept. Particles were characterized by Dynamic Light Scattering, zeta potential, Attenuated Total Reflection–Infrared Spectroscopy (ATR-IR), and scanning electron microscopy/energy X-ray spectroscopy. Besides bioaccumulation, toxic effects of MPs and NPs at different concentrations (12.5–200 mg/L) and in absence or presence of Cd (EC10 = 449.64 µg/L) and, separately, a mixture of Lindane/DDE (2000 and 25 µg/L, respectively) on RTgutGC were examined using a multiple endpoint viability assay, simultaneously measuring metabolic activity and lysosomal/cell membrane integrity. Characterization demonstrated that all particles present an incipient stability (-8 to -23 mV), except for the particles coated with Cd that rapidly aggregated (-1 to -12 mV). ATR-IR analyses indicated oxidation and modification of the surface chemistry after exposure to UV radiation. SEM analysis exposed that 6 weeks UV aging of the plastic particles notably altered its surface morphology, presenting more roughness and a damaged surface morphology. Data showed no toxicity on RTgutGC cell lines due to the combination of MPs/NPs with Cd or with the mixture of organics. The presence of MPs/NPs (25 mg/L) on the exposure medium indicated a reduction of cadmium and lindane/DDE bioavailability on RTgutGC cell lines. However, in the present investigation only acute exposure experiments were carried out, being unattainable to conclude that the presence of MPs/NPs cannot increase the toxicity and bioaccumulation of contaminants due to chronic exposure.

## **2.11.P-Tu080 Microplastics in Cold Water Corals Living up to 948 Meters Deep in an Irish Special Area of Conservation** **Alicia Mateos Cardenas, Aaron Lim and Andrew Wheeler, University College Cork, Ireland**

Microplastic pollution is ubiquitous, and marine systems have especially been studied for their presence. To date, microplastic studies on the deep sea have mainly focused on sediment cores and biota species such as fish and lobsters. Preliminary video data collected by a Remotely Operated Vehicle (ROV) from an earlier project led by the team previously showed that large plastic items are abundant, especially fishing items, in deep water Irish coral reefs from the Porcupine Bank Seabight, currently listed as Special Area of Conservation (SAC). However, there is a data gap in the presence of microplastics in cold water corals of such deep waters. In this study, we show for the first time the presence of microplastics in species of cold-water corals (*Leiopathes* sp. and *Lophelia pertusa*) collected at five sites at depths ranging from 893 to 948 m. Corals were examined on the surface of the coral skeleton and inside the stomach cavity of polyps after tissue digestion. The results from this novel study will be presented showing the different microplastic concentrations accumulated outside/inside the corals, a comparative analysis among species and any potential effects of environmental variables. Overall, cold water coral reefs are a habitat identified for monitoring under the EU Habitats Directive. Therefore, the results from this study will inform national (Irish) and European policy on ocean pollution.

## **2.11.P-Tu081 Sublethal Effects Induced by Different Nanoplastic Polymers to *Daphnia magna***

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A growing number of studies reports nanoplastics (NPs) capacity to generate potential hazardous effects on organisms, even higher than those induced by microplastics. The main criticisms of these works are related to the tested concentrations, which are higher than the typical environmental levels and the use of only polystyrene (PS).

In this perspective, this study aimed to evaluate the sublethal effects induced by three different NPs polymers (PS-NPs, polyethylene PE-NPs, and polyvinyl chloride PVC-NPs) of a nominal size of 200 nm, to establish whether their toxicity is comparable and if thus the effects of PS-NPs can be used as a proxy for all others.

For each polymer, five environmental relevant concentrations were tested (from 2.5 to 250 µg/L) on the freshwater cladoceran *Daphnia magna*, with 60 individuals exposed for 48 h to each concentration. NPs effects were assessed at the biochemical level investigating the amount of reactive oxygen species (ROS) and the activity of an antioxidant enzyme catalase (CAT), while at the individual level we evaluated the swimming response (distance moved).

Test results indicated that the exposure to different NPs polymers led to different biochemical and individual responses. The 48 h exposure to PS-NPs did not induce either oxidative stress conditions or alteration in the swimming behavior. Statistically significant changes (p-value < 0.05) in ROS levels and swimming distance were observed for PVC-NPs, where the higher concentration (250 µg/L) led to higher ROS content and induced an increased swimming stimulation. For PE-NPs treatment, a sharp decline in ROS was recorded for all the tested concentrations, while no effects were recorded at the individual level.

Our results highlighted that the exposure to environmentally relevant concentrations of NPs could pose sub-lethal effects to *D. magna*, and how these effects could differ with the different tested polymers. It is therefore recommended to conduct further studies on different NPs polymers, to better establish their different toxicity for the achievement of an NPs environmental risks assessment. In particular, the actual strategy to use PS-NPs as a proxy of the other plastic polymers seems not to be feasible, as polystyrene do not appear to be the most harmful NPs polymer.

## 2.11.P-Tu082 Constrasted Acute Effects of Nanoplastics on *Daphnia* and *Gammarus* Neonates in Comparing Natural Environmental Freshwaters

**Wei Liu<sup>1</sup>**, Zijiao Meng<sup>1</sup>, Rémi Recoura-Massaquant<sup>2</sup>, Arnaud Chaumot<sup>2</sup> and Serge Stoll<sup>1</sup>, (1)University of Geneva, Switzerland, (2)INRAE, France

By 2050, it is expected that 12 billion tons of used plastic will directly go to landfill or be discarded. Once in the environment plastic waste will be transformed into microplastic and nanoplastics (NPLs) via physical, chemical, and biological degradation processes. More concerning shows that nanoplastics are probably more widely distributed and more toxic than microplastics due to their larger surface-to-volume ratio and higher surface reactivity. Despite progress in evaluation of risk assessment, knowledge gaps largely exist understanding the toxicity of nanoplastics in aquatic systems considering nanoplastics surface properties, environmental media characteristics and species ecological traits. In this study, amidine-functionalized polystyrene nanoparticles (PS-NPLs) of different primary sizes of 20, 40, 60, and 100 nm are considered using lake water and mineral water to investigate the behavior and effects in neonate organisms of the plankton *Daphnia magna* and the benthos *Gammarus fossarum*. Key parameters including  $\zeta$ -potential, z-average diameter, conductivity, polydispersity index, pH, EC<sub>50</sub> were investigated to gain insight into the relationship between PS-NPLs intrinsic properties, their transformation behavior, water properties and species-specificity in the evaluation of PS-NPLs biological effects on crustacean neonates in natural aquatic environments. The results shown that PS-NPLs with different initial sizes induced a statistically significant mortality of *D. magna* and *G. fossarum* neonates with a dose-time response relationship, and exhibit 1.3-6.5 times higher toxicity in the mineral water than in the lake water. More importantly, ecotoxic effects are found driven by both the surface properties of PS-NPLs and environmental factors. The surface charge is also found the most influential toxicity factor of PS-NPLs on *D. magna* in environmental natural waters, while on *G. fossarum* the aggregation behavior is the stronger explanatory factor. The combination of the characterization of colloidal stability of nanoparticles and effect assessment in environmental natural waters systems in this work evidenced the relationship between colloidal stability of NPLs, exposure water properties, the physiological trait of organisms, and the biological response.

## 2.11.P-Tu083 Detection of Size-Dependent Physical Effects of Microplastics Using Extended Acute Toxicity Test

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In recent years, environmental effect of the microplastics (MP) are increasingly discussed and that ecotoxicity are becoming revealed. In our study, we exposed toxic chemicals to aquatic organisms with and without MP in a closed experimental water system to detect the difference in toxicity values, and the aim is to clarify if the adverse effects of MP exist. If a vector (chemical) effect exists, it is thought that the chemical would be transferred to the organisms more efficiently via MP than via waterborne exposure. We chose pyrene as the test substance, and spherical plastic beads (PB) made of polystyrene and polyethylene with various sizes from 0.2  $\mu$ m to 3 mm in diameter were used.

In a previous presentation, we showed the result that *Medaka* and *Daphnia* toxicity test (OECD TG 202 and 203) were conducted with pyrene under the presence or absence of PB. If the PB sizes were inedible to the organism, the toxicity was less with the presence of PB compared to absence of those. This may be because the pyrene was sorbed from water to PB and their concentration was decreased, resulting in lower exposure. In the case of the edible size of the PB, they were found in the digestive tract of organisms, but their toxicity remained the same with the exposure result only with pyrene. So no synergistic effects of toxicity due to vector effects were observed.

For the next step, based on the *Daphnia* Acute Toxicity Test (OECD TG202), we additionally continued the observation until the 96th hour with feeding after 48 h. If MP obstructs the gastrointestinal tract and inhibits nutrient absorption, it is thought that *Daphnia* will starve to death within 96 h even if the food is served. Seven types of PB with diameter of 100, 30, 10, 3, 1, 0.46, and 0.19  $\mu$ m were used for experiment to clarify the physical effects of MP. The PB solution was centrifuged 2 times to separate the medium since it includes dispersant, preservatives, and so on. The amount was weighed by dry weight. From the result, the toxicity of 100  $\mu$ m did not differ from the control even after 96 h. There was no acute toxicity with PB of the diameter 30, 10, 3, 1  $\mu$ m at 48 h, but the deaths probably due to starvation were observed after 96 h. Immobilization was observed with 0.46 and 0.19  $\mu$ m of PB due to adhesion to the body surface, etc.

It was confirmed that there were size-dependent difference in toxic effect of MP by using the test method of feeding and extending the exposure period.

## 2.11.P-Tu084 Comparison of Ecological Effects of a Diverse Mix of Microplastics with an Equally Diverse Mix of Inert Nonplastic Particles

**Vera de Ruijter**, Xinyi Xie and Albert A. Koelmans, Wageningen University & Research, Netherlands

Microplastics nowadays can be found throughout the aquatic environment. Consequently, they can be ingested by organisms and experimental work has shown that this could lead to potential negative effects. Many different kinds of negative effects have been reported including physiological stress, cell death, aberrant development, altered lipid metabolism, and intestinal damage. However, most negative effects reported include reduced body and population growth, or an energy reduction. In order to more accurately assess the ecological risk associated with microplastics, a better understanding of effect mechanisms and the key factors triggering them is needed. Currently, it is difficult to compare the impact of various factors on microplastic particle toxicity, as experimental setups differ significantly. However, literature review shows that most important factors determining the toxicity of microplastics include concentration, particle size, and exposure time. Additionally, important co-factors such as species and food availability were underlined. Contrastingly, much less is known about the effect mechanisms underlying adverse

effects. Most studies are not able to demonstrate the hypothesized effect mechanisms. While significant evidence exists for the mechanisms ‘internal physical damage (including abrasion of the gut) and external physical damage’, most evidence exists for “inhibited food assimilation and/or decreased nutritional value”, commonly referred to as ‘food dilution’. If this mechanism is important, any inert particle would be able to cause it, which is the hypothesis tested here. The model species *Lumbriculus variegatus* was used to compare the effects of an environmentally relevant microplastic mix (ERMP) on growth and survival, with those of a natural, non-polymer solid mix (NS) with equal polydispersity. This way, effects of MP are put in the context of natural particles. Moreover, using volume as an ecologically relevant metric in the dose response testing enables us to test the hypothesized effect mechanism food dilution. As the implications of food dilution are closely linked to food availability, the chronic dose-response tests with both particle types were performed at two levels of sediment organic matter.

#### 2.11.P-Tu085 eDNA Adsorption and Fate on Microplastics

*Nicolas David Müller, Anish Kirtane and Denise M Mitrano, Environmental Systems Science, ETH Zürich, Zürich, Switzerland*  
Microplastics (MPs) have been found in virtually every environmental compartment sampled to date. Consequently, there has been increasing research into processes and interactions MPs may undergo in the environment. Environmental DNA (eDNA) has similarly become increasingly discussed due to its uses in determining biodiversity levels and detection of invasive, endangered and rare species. Based on their environmental ubiquity, eDNA and MPs have a high interaction potential, and could be particularly relevant in scenarios with locally high MPs abundance, since MPs could promote long-range transport of eDNA throughout the environment. In these cases, MPs may act as vectors for genetic materials of interest, such as antibiotic resistant genes (ARGs), or DNA sequences that may give insight on MPs origin and transport paths throughout the environment. A better understanding of eDNA

adsorption rates to MPs and sorption isotherms would facilitate assessment of the role MPs play in acting as eDNA vectors. Here we investigated the adsorption of extracellular linear DNA onto MPs.

Three of the most environmentally prevalent polymers were examined; low-density Polyethylene (LDPE), Polyethylenterephthalat (PET), and Polystyrene (PS). Adsorption batch experiments were performed using purified linear extracellular salmon (*Oncorhynchus keta*) sperm DNA prepared in very hard and very soft synthetic freshwaters to assess the influences of water chemistry on adsorption over 24 h. Ionic strength was shown to impact DNA adsorption by electrostatic repulsion, which was reduced in hard water. PET exhibited the highest adsorption capacity when normalizing for MPs specific surface area, likely due to its hydrophilic surface chemistry and the presence of ester groups. Kinetics experiments exhibited fast adsorption during shorter incubation (under 30 min) before eventually reaching equilibrium adsorption capacity after 8 h. Isotherm experiments showed the equilibrium adsorption capacity was reached at DNA concentrations of 10-15 ng  $\mu\text{L}^{-1}$ . Overall, we demonstrated that DNA quickly binds to MPs and they could act as an effective vector for environmental genes of interest, such as ARGs, to be transported throughout the environment.

#### 2.11.P-Tu086 A Study on Species Sensitivity Distribution Approaches of Microplastics by using the Highest Observed No-Effect Concentration (HONEC) in Freshwater

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Predicted no-effect concentration (PNEC) of chemicals for protecting aquatic ecosystems is derived from the traditional toxicity values (e.g., no observed effect concentration, effective concentration at 10% or 50% effect, or the lowest observed effect concentration) based on guidelines for deriving water quality standards in Europe, Australia and New Zealand, and Canada. However, it is difficult to use only traditional toxicity values for microplastics because most studies have tested only one or two microplastic concentrations and have limited traditional toxicity values. Therefore, it is needed to consider non-traditional toxicity value to get more powerful toxicity data pool for microplastics. In this study, we collected a total of 398 chronic toxicity data of microplastics through various databases and tabulated for fish, invertebrates, algae, and macrophytes). The traditional toxicity values (NOEC and  $\text{E(L)C}_{50}$ ) and nontraditional toxicity values (HONEC) were 42% and 58%, respectively. The SSD curve resulted from the inclusion of the nontraditional toxicity values showed weaker HC values, good fitting, and similar spread distribution than the traditional toxicity values pool. We verified applicability of inclusion of the nontraditional toxicity value pool to derive hazardous concentrations for microplastic via inclusion of the nontraditional toxicity value pool.

#### 2.11.P-Tu087 Ecotoxicological Assessment of Microplastics from a New Plasmix-Based Material

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The development of new strategies to counteract the accumulation of plastics in the environment due to improper or inefficient disposal represents a priority in the waste management. Although most plastics are potentially recyclable, only 15% of the worldwide plastic wastes is currently recycled, while the remaining nonrecyclable part is landfilled or used for energy recovery. In Italy, this heterogeneous mixture of plastic wastes is called Plasmix. To date, Plasmix is landfilled or used for thermal recovery, leading to secondary pollution and loss of economic value for materials that could be reused or recycled. In recent years, we developed an innovative approach based on mechanical recycling aimed at modifying the Plasmix to generate a new Plasmix-based material to be used in the production process. Before being used, this material needs to be tested for its eco-safety because, once in the environment, it can interact with aquatic and terrestrial organisms, mainly after its degradation and/or fragmentation in microplastics. Thus, the present study aimed at evaluating the ingestion and the potential acute and chronic toxicity induced by exposure to different concentrations of microplastics derived from the fragmentation of a Plasmix-based

material, toward the freshwater Cladocera *Daphnia magna* and the earthworm *Eisenia fetida*. The results highlighted that both the model organisms efficiently ingested microplastics. In spite of the ingestion, no effects on Cladocera and earthworm survival occurred. However, a significant reduction of the reproductive success of *D. magna* treated individuals compared to the control group was noted. In addition, a decrease in body condition and the onset of oxidative stress situation occurred in earthworms. These findings suggest that microplastics generated by materials created by mechanical recycling of plastic waste can affect the health status of aquatic and terrestrial organism, confirming the crucial role of ecotoxicological analyses in the characterization of new materials.

## 2.11.V Filling Gaps for Micro- and Nanoplastic Effects and Risk in Multiple Stressed Aquatic Environments

### 2.11.V-01 *Asparagopsis armata* Exudate Combined with Virgin and Mercury-sorbed Polyethylene Microplastics: Histopathology and Byssal Thread Production of the Marine Mussel *Mytilus galloprovincialis*

Maria Donas-Bôto Bordalo<sup>1</sup>, Cristiana Lopes<sup>2</sup>, Andreia Rodrigues<sup>1</sup>, Sílvia Pires<sup>2</sup>, **Diana Campos<sup>3</sup>**, Francesca Coppola<sup>4</sup>, Tania Russo<sup>5</sup>, Gianluca Polese<sup>5</sup>, Amadeu M.V.M. Soares<sup>3</sup> and Hugo C. Vieira<sup>1</sup>, (1)CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal, (2)University of Aveiro, Aveiro, Portugal, (3)University of Aveiro & Centre for Environmental and Marine Studies (CESAM), Aveiro, Portugal, (4)Department of Biology & CESAM - University of Aveiro, Aveiro, Portugal, (5)University of Naples Federico II, Italy

Invasive species and microplastic pollution are two main threats to marine ecosystems. The red seaweed *Asparagopsis armata* is considered a successful invader in the European coasts and is known to exude toxic metabolites with recognized effects in different coastal organisms from cellular to individual levels and consequent implications at the population, community, and ecosystem levels. Microplastics are a significant part of marine debris and can be easily ingested by organisms from different trophic levels. Filter-feeders such as the bivalves are particularly prone to this source of pollution. Also, microplastics can carry several other contaminants (e.g., mercury - Hg), posing an additional impact to marine biota.

This study aimed to evaluate the effects of polyethylene microplastics (PE-MPs) either virgin (PE) or with sorbed Hg (PE-Hg) in the mussel *Mytilus galloprovincialis* under *A. armata* exudate exposure. Mussels were exposed to 6 treatments: control (no exudate nor PE/-Hg), *A. armata* exudate (4%), PE (1 mg/L), PE-Hg (1 mg/L), PE and exudate, PE-Hg and exudate. After 96 h, organisms were checked for the number of produced byssus as a measure of physiological response. Histopathological alterations were also analyzed in the digestive gland and gills. PE-MPs and Hg were quantified in the same tissues.

Microplastic particles were found mainly in the digestive gland, where consequently, the Hg concentration at PE-Hg treatment was also the highest. Although nonsignificant, the mean number of particles was lower in the presence of the exudate when compared to non-exudate-exposed organisms. In contrast, a significant increase in Hg concentration in the digestive gland was found in mussels exposed to the exudate and PE-Hg. Byssal thread production decreased significantly due to exudate exposure, irrespective of the PE-MPs treatment. On the other hand, histopathological results showed that mussels exposed to control treatment presented considerable differences compared to organisms exposed to PE-Hg and/or PE with exudate. These alterations were expressed as: i) cilia loss, hemocytes infiltration, and enlargement of the central vessel in the gills; ii) lipofuscin aggregates and hemocytes infiltration in the digestive tubules. These findings highlight the importance of evaluating the impacts of PE-MPs in the marine environment, and their combined effect with co-occurring stressors, which will be helpful for further evaluation of their ecological risk.

### 2.11.V-02 Responses of *Mytilus galloprovincialis* in a Multistressor Scenario: Effects of an Invasive Seaweed Exudate and Microplastic Pollution under Global Warming

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Coastal ecosystems are constantly under the influence of a variety of stressors, both natural and anthropogenic, with known consequences to the marine biota. Invasive species are considered a major threat to biodiversity. The red seaweed *Asparagopsis armata* is a highly invasive species, which exudes a cocktail of secondary metabolites as a defence mechanism, with known toxic effects on native organisms. Microplastic pollution is also recognized as an issue of growing concern. Polyethylene microplastics (PE-MP) represent the most common form of microplastic pollution in the marine environment. Given their ubiquity and bioavailability to marine organisms, MPs potentially affect filter-feeders such as the mussel *Mytilus galloprovincialis*. Being ectothermic, these bivalves are also under the threat of global warming. This study aimed to evaluate whether the toxic effects posed by *A. armata* exudate to *M. galloprovincialis* are amplified by the concomitant exposure to these stressors. Mussels were exposed for 96 h to 8 treatments, 4 at 20°C and 4 at 24°C: (i) control (absence of exudate and PE-MP), (ii) 2% *A. armata* exudate (2%), (iii) PE-MP (1 mg/L), (iv) PE-MP and exudate. Biochemical (oxidative stress, damage, and neurotoxicity biomarkers) and physiological (byssus production) parameters were evaluated. Mussels produced the lowest number of byssus under concomitant exposure to the three stressors. Decreased attachment capacity is critical not only at the individual but also at the community level, as the habitat complexity conferred by mussel beds may be compromised. Antioxidant responses were depleted in the gills of organisms exposed to PE-MP and increased temperature, irrespective of the exudate presence, being able to prevent oxidative damage in these scenarios. On the other hand, increased lipid peroxidation was observed in mussels exposed to exudate and warming but not PE-MP. Reduced protein carboxylation was observed in mussels only exposed to exudate. In the digestive gland, nonenzymatic antioxidant responses were activated in mussels simultaneously exposed to 24°C, exudate, and PE-MP, and

oxidative damage was prevented. Neurotoxicity (inhibition of acetylcholinesterase activity) was also observed in the same treatment. In conclusion, simultaneous exposure to these stressors does not necessarily mean an amplification of their single effects. Nevertheless, further studies should be conducted to better understand the mechanisms adjacent to these effects.

#### **2.11.V-03 Combined Effect of Microplastics and Ocean Acidification on Critical Stages of the Sea Urchin (*Paracentrotus Lividus*) Early Development**

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One of the major consequences of increasing atmospheric CO<sub>2</sub> is a phenomenon known as ocean acidification (OA). This alteration of water chemistry can modulate the impact on marine organisms of other contaminants present in the environment, such as microplastics (MP). The sea urchin *Paracentrotus lividus* is a good indicator of the status of the marine ecosystems, and since it needs to calcify its structures to grow and develop, is also a good model to study the impact of global climate change. The objective of this work was to determine the combined impact of MP and OA on the early development of *P. lividus*, and predict the impact of this particular mixture of contaminants/stressors on the marine environment. In a first set of experiments, sea urchin embryo tests (SET) were conducted to determine growth after 48 h of incubation with two concentrations of MP (1000 and 3000 particles/mL), and under several pH conditions. We found that the inhibition of growth in embryos reared at pH = 7.6 was around 75%. Larvae incubated at 3000 MP particles/mL showed around 20% decrease in growth compared to controls. In a second set of experiments, embryos were exposed to a control treatment (filtered natural seawater), MP (3000 particles/mL), acidified sea water (pH = 7.6), and a combination of MP and acidification (3000 particles/mL + pH = 7.6). After 48, 72, and 96 h measurements of growth and morphometric parameters were taken. Results showed that ocean acidification and MP cause alterations in growth and larval morphology both before and after the larvae start to feed exogenously (around 72 h). This work is part of a larger framework that pursues the utilization of SET for testing multistressor environments, particularly those including factors of global climate change. Through this approach, it has been demonstrated that OA and MP pollution can cause alterations in the morphology and growth of *P. lividus* larvae. The exposure to MP under conditions of OA did not produce an additional effect on growth, but differences were observed at the morphological level related to a decrease in the width of larvae at 48 h. Overall, changes in larvae shape observed at three key points of their development could modify their buoyancy affecting their ability to obtain and ingest food. Therefore, OA and MP pollution might compromise the chances of *P. lividus* to survive in the environment under future scenarios of global climate change, leading to a potential impact on coastal ecosystems.

#### **2.11.V-04 Implications of Single and Combined Acute Exposures to Microplastics and Naphthalene on the African catfish (*Clarias gariepinus*)**

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The toxicity of microplastics to aquatic organisms and their potential to induce the toxicity of other contaminants has received increasing attention. There are, however, still very few studies on the effects of microplastics in combination with other environmental contaminants like polycyclic aromatic hydrocarbons (PAHs). Therefore, the current study focused on the acute effects of single and combined exposures to microplastics and naphthalene on the African catfish (*Clarias gariepinus*). The effects of microplastic and naphthalene individually and in mixtures on survival, accumulation, as well as biochemical effects (Total protein and Acetylcholinesterase (AChE)) in the fish were assessed. Juveniles were individually exposed for 96 h to nine (9) treatments (control; contained only water), two concentrations of microplastics alone (0.5 mg/L and 2.0 mg/L (low-density polyethylene, LDPE), two concentrations of naphthalene (Nap) alone (20 mg/L and 50 mg/L), and four concentrations of a mixture of microplastics and naphthalene (20 mg/L naphthalene + 0.5 g/L LDPE, 20 mg/L naphthalene + 2.0 g/L LDPE, 50 mg/L naphthalene + 0.5 g/L LDPE and 50 mg/L naphthalene + 2.0 g/L). Results showed no significant differences ( $p > 0.05$ ) in the survival rate of the fish exposed to both the single and combined mixtures of microplastics and naphthalene, as nominal differences were observed between the control and treatment groups. Accumulation of the microplastics and naphthalene by the fish in the treatment groups was observed to be concentration dependent for both the single and combined mixtures. Results showed that the LDPE microplastics modulated the impact of naphthalene on the biochemical parameters assessed. The single exposure of *C. gariepinus* to naphthalene and LDPE microplastics, and the combined mixtures of LDPE with naphthalene, significantly inhibited acetylcholinesterase (AChE) activity compared to the control. The single and combined effect of naphthalene and LDPE microplastics induced neurotoxicological response in *C. gariepinus* after 96 h of exposure. Furthermore, the single exposure to naphthalene and the combined exposures also reduced the total protein concentrations in the fish species. These results, therefore, stress the need to further address the role of microplastics in the bioaccumulation and toxicity of other environmental contaminants in fish species.

#### **2.12 Municipal Wastewater: Proxy for Human and Environmental Exposure and Impacts on Soil and Aquatic Ecosystems**

##### **2.12.T-01 Emerging Contaminants in Wastewater – European Project on the Occurrence of Chemicals and Adverse Effects**

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Contamination of our freshwater systems by municipal wastewater carries the risk of adverse effects on aquatic organisms essential for a well-functioning ecosystem. Multiple wastewaters from private households, urban drains, hospitals, and industry containing chemicals of emerging concern, such as pharmaceuticals, personal care products, pesticides, hormones, and transformation products, are typically discharged via wastewater treatment plants into freshwater systems. In this study, sensitive analytical and bioanalytical methods and holistic assessment approaches have been applied to assess complex chemical mixtures in European effluents linking the occurrence of chemicals with adverse biological effects. A set of 56 treated wastewater samples from 15 European countries were investigated using:

1. Chemical target screening of 499 chemicals to identify and prioritise compounds of concern, comparing three different toxic risk assessment metrics (Risk Quotient, Toxic Unit, Hazard Unit).
2. *In vitro* effect-based methods of four cellular endpoints (estrogen-, androgen-, glucocorticoid-, and progesterone receptors) linked with chemical analysis of 79 steroids and phenols to identify and assess endocrine disrupting chemicals.
3. *In vivo* effect-based methods on whole organisms (i.e., growth and PSII inhibition, immobilisation, and mortality) to investigate adverse effects on algae, crustacean and fish.

In the effluent samples 366 chemicals of emerging concern and 42 endocrine disrupting chemicals were detected at concentrations from <1 ng/L to >100 µg/L. Receptor activities were detected in between 14 % and 82 % of the samples, while apical effects (EC<sub>50</sub> from the concentration-response curve) were measured in 96 % (algae), 51 % (daphnia) and 82 % (fish embryo) of the samples. For endocrine disruptors, measured and predicted bioanalytical equivalent concentrations (BEQ<sub>bio</sub> and BEQ<sub>chem</sub>, respectively) were highly correlated and allowed the identification of effect drivers. In summary, integrated monitoring methods and mixture assessment tools indicated a substantial risk of receiving waters from wastewater treatment plant effluents and a substantial mitigation by advanced treatment technologies. Major risk drivers were identified supporting chemical regulation and measures at the source. The integrated approach may be used as a blueprint for future water quality monitoring. One of the bottlenecks is still the lack of measured effect data for major wastewater contaminants.

## 2.12.T-02 Changes in Chemicals of Emerging Concern in London's Rivers Across the SARS-CoV-2 Pandemic

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The SARS-CoV-2 pandemic has had a huge impact on global urban populations, their activity, and health. However, little is known about impacts of such a public health crisis on urban river ecosystems. The aim of this work was to determine whether significant changes in chemicals of emerging concern (CECs) occurred in rivers located in Greater London (UK) across the pandemic. The objectives were to (a) reliably and rapidly identify and monitor large numbers of CECs at n = 390 sites across 2019, 2020 (during lockdown), and 2021 using two novel direct-injection liquid chromatography mass spectrometry methods for targeted and suspect screening and (b) to interpret changes in CEC concentrations trends and risks in relation to public health data. Out of all 164 CECs monitored, 66 CECs were quantified across all sites using targeted analysis. Pharmaceutical measured environmental concentrations (MECs) decreased during the SARS-CoV-2 lockdown period in 2020 in the R. Thames (p<0.001), but then increased to higher MECs in 2021 (p<0.01). Across all three years, MECs rose for some antidepressants and antipsychotics and aligned with National Health Service (NHS) medicine prescription data. For the tributary rivers, the R. Hogsmill, Lee, and Wandle were the most impacted across their lengths and predominantly by WWTP discharge. For the R. Hogsmill in particular, it was observed that prescribed pharmaceutical MEC trends generally associated with NHS statistics due to low dilution. Suspect screening based on a library of >1200 compounds revealed a total of 25 additional compounds at the five most-impacted sites. This included several metabolites, for example, high frequency detection of O-desmethylvenlafaxine. Lastly, risk quotients (RQ)>0.1 were calculated for 26 compounds. The highest RQs were for imidacloprid which is mainly used in the UK as a pet parasiticide (medium-high risk at wastewater effluent discharge sites). Selected medications such as antipsychotics and antidepressants also rose into higher risk categories. The overall impact of the SARS-CoV-2 pandemic was multifaceted, with short-term changes in CECs observed in rivers during lockdown periods, but impacts were also observed beyond this. With over 10 029 quantifiable CEC occurrences across all samples, this is the largest dataset of its kind both spatially and temporally for Greater London, representing ~16 % of the population of England and a useful case study of the third largest city in Europe.

## 2.12.T-03 Wastewater-Based Epidemiology to Assess Human Exposure to Multiple Factors Affecting Human Health

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Wastewater-based epidemiology (WBE) is an innovative methodology enabling the retrieval of epidemiological information from



urban wastewater via the chemical or biological analysis of specific human metabolic excretion products (biomarkers). WBE may provide information at the community level on emerging risky lifestyle habits, the circulation of pathogens (viruses and bacteria), the exposure to contaminants, and the health status. This study aimed to assess multiple factors that may seriously affect human health using WBE. The factors investigated were: licit and illicit drugs use (prescription opioids, new psychoactive substances - NPS); antibiotics use related to antimicrobial resistance. Specific substances were selected according to the requirements requested for applying a WBE approach and were validated as WBE biomarkers. Urban wastewater samples were collected at the inlet of wastewater treatment plants as composite 24 h samples, and were analysed through solid phase extraction and liquid chromatography coupled to tandem mass spectrometry. All analytical methods were validated according to current guidelines and performed well for sensitivity and selectivity. For prescription opioids, human consumption was estimated from mass loads (mg/day/1000 inhabitants) by using specific correction factors that were developed within the present study and took into account urinary excretion rates. Results showed different profiles of use in the north and south of Italy for some substances (e.g., tramadol). The comparison with prescription figures showed a quite good agreement, suggesting that in Italy there is no abuse of prescription opioids as observed in other countries. A list of 43 “priority NPS” was selected for monitoring and 8 were detected in Italy and Europe. Changing profiles of use were found compared to previous studies with an upsurge of the synthetic cathinone 3-methylmethcathinone. The main classes of antibiotics were investigated in urban wastewater influents highlighting the most abundant classes that were fluorochinolones, macrolides, sulfonamides, and tetracyclines. WBE was also applied to follow azithromycin use during COVID-19 pandemic and explore its potential abuse. WBE was a very useful approach to obtain a wide range of information on the health status of a population and was able to identify public health emergencies as the high use of antibiotics and track rapidly emerging risky lifestyle habits as the abuse of new psychoactive substances.

## **2.12.T-04 Is it Safe to Irrigate Fresh Produce with Reclaimed Wastewater? Evidence from Human Exposure and Risk Assessment Study**

**Benny Chefetz**, *Jorge Tarchitzky, Vered Mordehay and Evyatar Ben Mordechay, Hebrew University of Jerusalem, Israel*  
Irrigation with reclaimed wastewater is a growing agricultural practice. However, this practice exposes the agricultural ecosystem to organic pollutants that were not completely removed during the wastewater treatment. Once introduced into the field these pollutants may be taken up by plants and introduced into the food chain. The current study used Israel as a case study to estimate the unintentional exposure of the population to organic pollutants originating from wastewater. We used up-to-date dietary data and measured the concentrations of organic pollutants in produce representing ~75% of the diet. Human health concerns were estimated using two approaches: the acceptable daily intake (ADI) and the threshold of toxicological concern (TTC).

Leafy vegetables exhibited the highest concentration of organic pollutants; thus the highest human exposure to the wastewater-borne pollutants was for population subgroups consuming high amounts of leafy vegetables, such as vegetarians and Israeli Arabs. For the extreme exposure scenario (calculated as maximum contaminant concentration times the 95th percentile consumption), the anticonvulsant drugs Lamisil, Tegretol, and a therapeutically active metabolite of Tegretol exhibited the highest human exposure levels of 29.1, 27.2, and 19.5 micrograms per person per day, respectively. For the general population and using an average exposure scenario, the above-mentioned anticonvulsant drugs exhibited exposure levels below the ADI and TTC thresholds. However, for the extreme scenario, the exposure level of Tegretol was higher than its ADI level. In addition, the maximum exposure levels of Lamisil and the therapeutically active metabolite of Tegretol were higher than the TTC level for genotoxic compounds.

To reduce the potential risk, it is suggested to exclude leafy vegetables from agricultural water reuse, and/or improve wastewater treatment to completely remove the anticonvulsant drugs Lamisil and Tegretol.

## **2.12.T-05 Circularity in the Olive Mill Wastewater Management: Evaluation of the Wastewater Treatment From an Ecotoxicological Perspective**

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Olive oil is widespread in Mediterranean cuisine and represents an ecological challenge as its manufacture entails the generation of vast amounts of liquid and solid waste. Liquid waste – olive mill wastewater (OMWW) – has long been a concern for all olive oil producers. Many wastewater treatment methods have been used, yet no universal solution exists.

The chemical precipitation technique is a novel wastewater treatment method, using a single reagent – lime – that reacts with atmospheric CO<sub>2</sub>, resulting in ionic precipitation. This technique will originate treated wastewater and a precipitate (sludge) that might be used mainly in soil amendment. Simultaneously, wastewater can be reused in irrigation, hydroponics, etc. However, the ecotoxicological weight of CPT-treated wastewater (CPT-OMWW) and sludges has yet to be deeply understood, as little work has been carried out. This work aimed to assess the efficacy of CPT for treating wastewater and whether the sludge is

ecotoxicologically safe for soil application. For that, a battery of aquatic and soil ecotoxicological assays was performed in both CPT-OMWW and corresponding sludges.

For the ecotoxicological evaluation of both OMWW and CPT-OMWW, four aquatic species (*Raphidocelis subcapitata*, *Daphnia magna*, *Danio rerio*, *Lumbriculus variegatus*) were used. Acute endpoints were obtained for each species, and LC<sub>50</sub> and EC<sub>10</sub> values were estimated depending on the species. CPT reduced the toxicity of *D. magna* and *D. rerio* (12.7% and 13.89% LC<sub>50</sub> OMWW to almost 100% survival at all tested concentrations after CPT). Likewise, *R. subcapitata*'s EC<sub>10</sub> (for average daily growth rate (Day<sup>-1</sup>)) increased after CPT treatment (2.95% to 26%). *L. variegatus* showed no toxicity for OMWW, and that no-toxic pattern continued after CPT treatment.

For the ecotoxicological sludge evaluation, four species (two invertebrates: *Folsomia candida* and *Enchytraeus crypticus*, and two plants: *Lolium perenne* and *Brassica oleracea*) were used. Sludge was mixed with LUFA 2.2 soil at 0, 0.5, 1, 2, 4, and 8% concentrations. Significant differences from the negative control – no sludge application – were estimated. Sludge did not affect either *F. candida* or *E. crypticus* adult survival at any tested concentration, yet there was a decrease in reproduction for both at 2% and 1% concentrations compared to control. Sludge did not affect seedling (Germination Index) in either of the tested plant species but, in general, decreased shoot and root biomass in both.

## **2.12.P Municipal Wastewater: Proxy for Human and Environmental Exposure and Impacts on Soil and Aquatic Ecosystems**

### **2.12.P-Tu088 The First National Scale Evaluation of Total Nitrogen Stocks and Burial Rates of Coastal Sediments Along the West, South, and East Coast of South Korea**

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The regulating ecosystem services, such as water purification, etc., that tidal flats provide by nitrogen (N) burial are being increasingly recognized; yet, quantitative estimates remain limited. Here, we first present a nationwide evaluation of total N stocks and burial rates in the Korean tidal flats, based on three-year-long monitoring assessment combined with a remote sensing approach. A total of 20 intertidal flats representing 7 provinces of South Korea were extensively surveyed in 2018–20; West Sea (Gyeonggi, Chungnam, Jeonbuk, and western Jeonnam), South Sea (southern Jeonnam and Gyeongnam), and East Sea (Gyeongbuk and Gangwon). The sediment textural type (sand, mixed, and mud) classified from remotely sensed imagery was significantly correlated to that identified from field data ( $p < 0.01$ ), warranting a nationwide estimate of total N stocks. The estimated total N stocks and burial rates in the tidal flats of Korea were 1.5 Tg N and 7,805 Mg N yr<sup>-1</sup>, respectively. Total N stocks significantly varied by region, province, morphology, salinity, and land use type adjacent to the corresponding tidal flats. In general, the N stocks of tidal flats seemed to be influenced by the degree of terrestrial N inputs to the ocean, whilst the N stocks were significantly correlated with several physical parameters, such as precipitation ( $p < 0.05$ ) and tide ( $p < 0.01$ ). Sediment properties such as mud content (%) were found to be the key factor in determining the N stocks across 20 intertidal flats ( $p < 0.01$ ). Finally, the economic value of the total N removal was estimated as ~222 million USD yr<sup>-1</sup> in Korea and ~15 billion USD yr<sup>-1</sup> globally. Overall, the present work confirms the valuable ecosystem services of tidal flats' cost-efficient N removal capacity, highlighting marine ecosystem services.

### **2.12.P-Tu089 Health Hazard in the Baltic Sea: The Presence of Total Fluorinated Substances in Various Matrices**

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Per- and polyfluoroalkyl substances (PFAS) is a collective name for approximately 5000 synthetic chemicals used in a myriad of products. Their commonness results in large exposure to humans and the environment. PFAS have been associated with cancer, developmental toxicity, immunotoxicity, endocrine disruption, and other health effects. Only a few PFAS have been studied and the currently regulated and monitored are only a small fraction of the total PFAS released into the environment. Wastewater treatment plants (WWTP) represent an important source of marine PFAS pollution. The Baltic Sea is considered a vulnerable ecosystem with low resilience, mainly due to the low number of key species dominating the ecosystem as well as the high level of contamination including PFAS. The aim of this study is to measure the specific concentration by target analysis of a large number of selected conventional and emerging PFAS, at two sites in the Baltic Sea in the vicinity of WWTPs, i.e., the archipelago south of Stockholm and Himmerfjärdens WWTP and in Gdansk the WWTP of Gdansk. PFAS will be measured up-stream the WWTP, at the outlet and in a gradient from the outlet, and in different matrices including water, sediment, plankton, and organisms in Baltic Sea food web. The target analysis will be complemented by measuring extractable organic fluorine (EOF) which will indicate the total amount of PFAS in the environment.

### **2.12.P-Tu090 Anthropogenic Gadolinium and Pharmaceuticals Along River Rhine From its Alpine Source to the Middle Rhine Region**

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Gadolinium (Gd) and pharmaceuticals have become a vital part of our everyday life. Gd applications range from high-tech electronics to medical applications, such as magnetic resonance imaging (MRI). MRI contrast agents based on Gd have been used since the late 1980s and have become important for medical diagnostics. After application, the contrast agent (with Gd) is

excreted nonmetabolized with urine and cannot be effectively removed from wastewater by sewage treatment plants. As a result, surface waters in medically advanced countries exhibit anthropogenic generated Gd anomalies (Gd<sub>anth</sub>), which can be detected even in drinking water. Long-term and side effects of Gd are under discussion. The most commonly detected pharmaceuticals, such as diclofenac (pain reliever) and carbamazepine (anti-epileptics), show a similar environmental transport pattern. The River Rhine is an important drinking water reservoir in Europe; water quality research is focusing mainly on the lower river part.

In this research, we investigated the occurrence of Gd<sub>anth</sub> and pharmaceuticals in Rhine river water from its source to the middle Rhine Region in Germany. In addition, water samples were taken from the most important tributary rivers Aare, Neckar, Main, Nahe, Lahn, and Mosel. All samples (total number 38) were taken within 9 days (mostly within 3 days) during a dry period in September 2021 and hydrochemically characterized by on-site parameters and ion-chromatography. Quantitative analyses of Gd<sub>anth</sub> were performed by ICP-MS (total Gd) and pharmaceuticals by solid phase extraction, derivatization, and GC-MS analyses. The hydrochemical development of the Rhine water reflected the geological background of the catchment and tributary catchments. From all samples only the Rhine source, Lake Toma, was free of Gd<sub>anth</sub>. Along the River Rhine remarkable peaks of Gd<sub>anth</sub> occurred at the river mouth of Neckar, Nahe, Lahn, and Mosel. A strong positive correlation between Gd<sub>anth</sub> and diclofenac ( $R^2 = 0.83$ ) as well as Gd<sub>anth</sub> and carbamazepine ( $R^2 = 0.81$ ) can be observed, indicating that both Gd and pharmaceuticals may have a common source. This is supported by a correlation between Gd<sub>anth</sub> and the respective population size. In general, the Rhine water load increased from the river mouth Aare to the confluence Mosel/Rhine more than 10-fold and reached a load of maximum ca. 2955 kg/a.

#### **2.12.P-Tu091 Monitoring the Metal Particulate in Municipal Wastewater Treatment Plant**

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Due to the development of nanotechnology, particulates are used in various industrial and household chemical products. Due to the increased use of particulates such as nanomaterials, the inflow of particulates into sewage is gradually increasing, raising concerns that environmental exposure of fine particles will cause serious harm to human health and ecosystems. In this study, the treatment efficiency of particulate pollutants was evaluated for each treatment process at three municipal wastewater treatment plants with different treatment processes (A2O, NPR, SBR). The samples were taken from influent, primary treatment, bioreactor, total phosphorus coagulation, filtration, UV disinfection, and effluent. Metal concentrations of 14 elements (Ag, Al, Au, Ca, Cd, Ce, Co, Cu, Fe, Mg, Mn, Ni, Ti, Zn) were measured in the sample. Total metal concentrations were analyzed with acid microwave digestion followed by ICP-MS or ICP-OES. Truly dissolved metals were separated using 10kDa ultracentrifugal filtration and the filtrates were analyzed by ICP-MS. Particulate metals were estimated by subtracting the truly dissolved metals from total metals. Sub micro(<μm) size metal particles were analyzed using spICP-MS to obtain the particle size and concentration. Chelex-100 resin, ion exchange resin, were used to remove excess ions from samples which may hinder the distinction of particle signals from background or ion signal resulting in the high BED (background equivalent diameter). The municipal wastewater treatment plant with A2O treatment method removed 96.2% of particulates in the bioreactor process. The municipal wastewater treatment plant with NPR treatment method removed 81.5% of particulates in the total phosphorus coagulation process. The municipal wastewater treatment plant with SBR treatment method removed 49.5% of particulates in the total phosphorus coagulation process.

#### **2.12.P-Tu092 Where Does All the Salt Come From: Sources and Dynamics of Freshwater Salinisation in German Rivers**

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Excessive salinisation of water resources poses a risk to humans and reduces aquatic biodiversity. Salinisation of waterways is understood to have both anthropogenic (e.g., agriculture, mining, road de-icing, wastewater treatment, and industrial discharge) and natural drivers (e.g., geological weathering products, atmospheric salt deposition, and natural saltwater intrusion). Furthermore, climatic conditions affect the volume and dilution capacity of rivers, leading to variations in salt loads.

The magnitude to which of each of these drivers contributes to the salinity of European water bodies is not well understood. In particular, it has not yet been studied how much the increasing amount of metal salts now being used in the wastewater treatment process to reduce phosphate loads, which is one of the goals of the Water Framework Directive, contributes to freshwater salinisation.

To address these knowledge gaps, we compiled a dataset from routine state monitoring programs across all of Germany, covering the 2000 to 2022 timeframe. Using time series analysis, we explored the spatial distribution and temporal development of salinity-related parameters (e.g., electrical conductivity and concentration of salt ions) and related these observations to potential drivers of salinisation (e.g., geology, location of wastewater treatment plants, proximity of roads) using regression models. In order to clarify whether wastewater treatment plants and especially phosphate elimination are significant drivers of salinity, we added hand-collected monitoring data from three waste water treatment plants we monitored for one year.

Our work improves the knowledge of the relative, quantitative contributions of the different drivers to the salinity of German limnic waters, which is crucial for informing regulations and identifying countermeasures. Moreover, our study demonstrates the

power of merging monitoring data across states and institutions, serving as an argument for redoubling data standardisation efforts.

#### **2.12.P-Tu093 Possible Future Applications for Nationwide Wastewater Surveillance in the Netherlands**

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From the onset of the COVID-19 pandemic in early 2020, a monitoring program for the presence of SARS-COV-2 in domestic sewage influents was set up in the Netherlands. Since September 2020, all 312 Dutch Sewage Treatment Plants were sampled; since November 2021 even 4 times a week, and subsamples are biobanked. Surveillance data are used, next to the results of other surveillance programs, to underpin policy measures. This established infrastructure is the result of a close cooperation in the Netherlands between the Ministry of Health, Sport and Welfare, the National Institute of Public Health and the Environment, and all 21 regional water authorities and is to our knowledge unique in the world.

Now that this infrastructure is in place, it gives the opportunity to monitor all kinds of both biological and chemical parameters, next to SARS-COV-2, on one side reflecting the health status and exposure of the population connected, and on the other hand revealing relevant information regarding compound groups affecting our water quality. Prominent parameters are infectious diseases like polio and influenza, antibiotic resistance, illicit and illegal drug use, and contaminants of emerging concern like PFAS. But which factors are the most relevant in the process of adding parameters to the existing wastewater surveillance? In this poster the potential parameters to be included in the wastewater surveillance are discussed, based on extensive literature study and expert consultations for the different goals and parameters. In this analysis the most important aspects involved are the added value, prospects for action, and ethical and legislative issues.

#### **2.12.P-Tu094 Hazard Screening of Contaminants of Emerging Concern (CECs) in Swedish Surface Waters**

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Persistent, mobile, and toxic substances (PMTs) have recently garnered increased attention by environmental researchers, the water sector, and environmental protection agencies. One particular concern for the environment is the unknown transport and spatial distribution of PMTs, which can result in PMTs causing toxic effects far from their source. Both assessment factor (AF) and species sensitivity distribution (SSD) methods can be used in component-based methods (CBMs) for environmental hazard assessment (EHA). AF methods are often limited to a few taxonomic groups, thus rarely including all taxonomic groups that have shown occurrence decline in freshwater ecosystems. A relaxed SSD approach can be used in a CBM EHA using all available ecotoxicity data without requirement of a certain number of taxonomic groups. In this study, acute and chronic SSDs were retrieved from literature data for previously quantified contaminants of emerging concern (CECs) in Swedish surface waters ( $n = 92$ ) and risk quotients (RQ) were calculated. Moreover, to better understand the characteristics of the detected CECs in nonurban lake sites ( $n = 71$ ), these compounds were checked against established criteria for PM(T)s and occurrence in the aquatic environment, respectively. PMT criteria were limited to results after 2017, as that year the definitions were updated by expert authorities. For the CECs with missing SSDs ( $n = 15$  [acute],  $n = 41$  [chronic]), ecotoxicity data were extracted for eight taxonomic groups, and if data were sufficient ( $n \geq 3$ ), SSDs were derived. AFs were used when the ecotoxicity data were insufficient. The retrieved and newly developed SSDs were then used in an EHA in the investigated Swedish rivers and lakes. In the rivers, 8 CECs had  $RQ > 1$  in at least one location, and 20 CECs posed a moderate risk ( $0.01 < RQ < 1$ ). Five CECs exceeded 'no risk to the environment' in the lake samples. In total, 21 of the 71 detected substances had already been identified as PM(T)/vPvM substances. The remaining CECs were examined for the fulfillment of the PM(T)/vPvM criteria by monitoring data. Detection frequencies (DFs) of previously identified PM(T) substances were compared with CECs within a similar DF range. 16 PM(T)/vPvM substances were detected in nonurban lake samples, and another 7 potential PM(T)s/vPvM substances were detected. Overall, our study shows the importance of studying field data at large spatial scale to reveal potential environmental hazards far from source areas.

#### **2.12.P-Tu095 Emission Estimation of Pharmaceuticals to Wastewater in Urban Areas – Is the Devil in the Detail?**

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With increasing numbers of chemicals used in modern society, assessing human and environmental exposure to them is becoming increasingly difficult. For example, measuring all >26 000 chemicals registered under REACH is not just technically unfeasible but would also be incredibly expensive. Estimating emissions of chemicals based on production and usage data could offer a more efficient and systematic approach to tackle this issue. However, such modelling exercises typically depend heavily on the quality of the input data. Currently, the optimum level of detail for input data required to derive sound emission estimations is unclear. Compared to other groups of CECs, data on the use of pharmaceuticals are relatively abundant and openly accessible, particularly for prescription drugs used by the general public. We assessed the implications of using different levels of detail in consumption data of 27 active pharmaceutical ingredients (APIs). The dataset used allowed for specification between national and local data, annual and monthly data, data on prescriptions, and usage in health care institutions. For selected pharmaceuticals, also the contribution of over-the-counter sales was evaluated. Furthermore, different routes of administration were considered as well as

the excretion of conjugate metabolites. For some APIs substantial differences in annual per capita prescriptions between local and national scale were observed. Intra-annual variability in local prescription data varied between 5% and 104%. Overall, the general public uses >90% of the total mass of all studied APIs, of which 20% via prescriptions and 80% via OTC sales. Emission estimates were compared to field measurements obtained through passive sampling. Sets of Speedisks were deployed at various locations throughout the municipal sewer system, covering a diverse selection of urban emission sources, including a hospital outlet, elderly home, residential area, student complex, and the local wastewater treatment plant (WWTP). Despite the variability in consumption, field measurements are subject to larger variations in time than emission estimates. When accounting for the excretion of glucuronide metabolites, emission estimations improved substantially even at locations 'upstream' to the WWTP, indicating rapid deconjugation and retransformation into the respective parent compound.

## **2.12.P-Tu096 Identification of Pharmaceutical Metabolites by Target and Suspect Screening in WWTPs and Their Abatement During Wastewater Treatment**

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Humans are exposed to a multitude of chemical compounds such as pharmaceuticals, which are upon intake often metabolized in order to increase polarity and facilitate their excretion. Detection of these metabolites in the influent of wastewater treatment plants (WWTPs) can give information on the exposure of a large fraction of the human population, whereas the detection in the effluent can yield indications about the pollution of aquatic systems. Many human metabolites arrive at WWTPs in higher amounts compared to their parents and are in some cases known to have higher toxicity. Nevertheless, many studies exist which analyze the parent compounds or focus on already identified metabolites of single compounds. Therefore, this study aims to identify and analyze metabolites originating from a broad range of pharmaceutical parent compounds by online-SPE-LC-ESI-HRMS/MS in combination with target and suspect screening. For this purpose, untreated wastewater of three Swiss WWTPs equipped with ozonation as a tertiary treatment was sampled for one week in early 2022 during dry weather conditions. The suspect list was generated based on the yearly consumption amounts of parent pharmaceutical compounds in Switzerland. Pharmaceuticals with a consumption amount of more than 100 kg per year or a strongly increasing consumption over the last few years were chosen, yielding 250 parent compounds. To complete the suspect list with the respective human metabolites, literature search, mainly with drugbank and Swiss compendium, was performed. The resulting list encompasses around 1100 metabolites of which 100 were covered by a targeted approach. Feature detection and data analysis were performed by SLAW and R, leading to the identification of several pharmaceutical metabolite compounds with confidence level 2 to 3. In addition to the raw wastewater, samples after every treatment step in the WWTPs were taken to assess the different abatement of parent and respective metabolites. It is anticipated that human phase I metabolites are less efficiently abated during oxidative biological wastewater treatment and ozonation compared to their parents, since they are already oxidized during human metabolism, such that they will be less efficiently attacked by the electrophile oxygen. The results of the ongoing study with focus on metabolites will be presented.

## **2.12.P-Tu097 Effect-Based Evaluation of Water Quality in a System of Indirect Reuse of Wastewater for Drinking Water Production**

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Freshwater resources are globally under an ever-growing pressure due to high demand and low supply. Furthermore, it is well known how low-level pollution of organic micropollutants can affect freshwater sources. Treated wastewater is often described as point source for this type of pollution. Using treated wastewater as a direct or indirect (via surface water) source for drinking water production therefore comes with a paired risk of presence of chemical pollution as well as presence of pathogenic microbial pollution. In drinking water production, disinfection is a well-established method to combat microbial pollution. It is however known that such treatment can cause formation of disinfection by-products (DBPs) which can induce oxidative stress. In this study, chlorination as a means of disinfection was applied to effluent wastewater indirectly used for drinking water production.

Water quality was assessed for the presence of bioactive compounds in a system of intentional reuse of effluent wastewater in and around the Llobregat River, Barcelona, Spain. Grab water samples included different stages of treated wastewater, recipient surface water, and finished drinking water. Samples were collected in two campaigns, with and without chlorination treatment applied to effluent wastewater. Water quality was assessed in a battery of reporter gene assays using stably transfected mammalian cell lines. Endpoints included estrogen and androgen receptor activity, AhR activity, immune response via NF- $\kappa$ B activity as well as oxidative stress, via the Nrf2 pathway, as a measure of formation of DBPs.

Our results indicate that in this investigated setup, effluent wastewater could be purposely redirected to increase freshwater supply for drinking water production without compromising the quality of finished drinking water. Furthermore, we found chlorination treatment of effluent wastewater to result in three major findings. First, we could not observe an increase in oxidative stress response due to the additional chlorination treatment. Secondly, estrogenic agonistic activity decreased after chlorination treatment and thirdly, we found AhR activity to increase after chlorination treatment.

Some of the results in this study need further investigation to reveal mechanisms behind the specific findings. This study provides important insights for future efforts to increase reuse of treated wastewater as a source for drinking water production.

#### **2.12.P-Tu098 Nicotine in Coastal Waters of the Iberian Peninsula: Environmental Risk and Possible Use as an Indicator of Bathing Water Quality**

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Densely populated coastal areas are highly vulnerable to pollutants from daily human activities. An increasing number of contaminants have been detected in coastal waters highlighting the need to better understand their possible risks. Among these, nicotine has been proposed as a potential marker of anthropogenic contamination due to its ubiquitous presence in coastal areas. Currently, quality of bathing water quality regulations in Europe are only based on microbiological parameters according to Directive 2006/7/EC. The objectives of this study were (1) to determine the presence of nicotine and its main metabolite, cotinine, in three different coastal areas in the south of the Iberian Peninsula, (2) to determine their environmental risks, and (3) to evaluate their usefulness as a water quality indicator. Samples were collected and analysed by liquid chromatography (HPLC) along 16 beaches in the Portugal coast in Lisbon/Algarve, and 30 in Spain: 18 from Cádiz and 12 from Mar Menor (Murcia). The water quality, according to National Bathing Water Information System (Spain) and National Water Resources Information System (Portugal), was evaluated as excellent in 100% of the beaches. Nonetheless, nicotine and cotinine were detected in 97% and 100% of the samples, respectively. For the risk assessment, available chronic toxicity data provided by ECOSAR for both substances were used to calculate the hazard quotient (HQ). To determine the relationship between the microbiological parameters and the concentrations of nicotine/cotinine an analysis of non-parametric contrasts for comparison of medians was carried out. Of all the samples, Veneciola beach in Mar Menor showed the highest hazard (HQ=5) for nicotine, while no risk of cotinine (HQ<0.1) was detected in any area. For nicotine, 14% of the samples presented no risk, 27% low risk and 59% reached moderate risk. No correlation was found between the numbers of bacteria detected and nicotine/cotinine concentrations. This study demonstrates that nicotine and cotinine are ubiquitous contaminants in densely populated beaches, which most likely come from wastewater treatment plant effluents, but also from cigarette butts and human excretion. The risks posed by nicotine can reach high values in peak season. Therefore, further studies should investigate the subchronic and chronic effects of this substance on marine organisms.

#### **2.12.P-Tu099 Characterization of Biological Communities in Systems Affected by Wastewater Treatment Plant (WWTP) Effluent Discharge**

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Wastewater treatment plants (WWTP) are essential to modern day life; they play a key role in reducing the environmental impact of everyday human activities. However, because WWTPs were mainly designed to reduce organic matter load and disinfect the influent, it is frequent that released treated effluents still bear some contaminants. Whereas most of the mandatory quality assessment standards that WWTPs should meet focus the water compartment (effluent and/or recipient waterway), some of those contaminants might accumulate in the sediment and have an impact in benthic communities in the vicinity of the effluent discharge. Benthic macroinvertebrate and diatom communities are models in this context while also being biological elements used for evaluation of ecological quality under the scope of the European Water Framework Directive (WFD). Herein, we aimed at evaluating the impact (if any) of the effluent in both these communities, learn how their structure changes with distance from effluent discharge, and compare the outcomes of community structure analysis with ecological status classification to appraise on the relative sensitivity of both methods in highlighting responses to effluent-driven contamination. We collected macroinvertebrate and diatom samples, as well as sediment samples for multiresidue analysis, at different distances from effluent discharges in the recipient waterways of three WWTP: immediately upstream the effluent discharge point, immediately downstream the effluent discharge point, and 500 m downstream from the discharge point. Results showed that effluent sourced contaminants accumulated in the sediment (e.g., pharmaceuticals and metals), but also that links to negative impacts in biological communities are not consistently recognized, denoting that the effluent may not be the single most effective stressor constraining communities. A discrepancy was found between the ecological status classification determined on the basis of one or the other community, up to two classification categories, which suggests a high level of specificity of this stressor scenario and highlights the need for a careful selection of biological elements to focus in WFD biomonitoring efforts.

#### **2.12.P-Tu100 Effects of the Antimicrobial Agent Thymol on the Physiological Profile of River Microbiota**

*Cristina Gan-Oria, Elisa Langa, Antonio Valenzuela, Natalia Ferrando Lázaro, Guillermo Lorca, Diego Ballesteros and M. Rosa Pino, Universidad San Jorge, Spain*

Thymol is a natural product derived from the secondary metabolism of plants, being the main monoterpene phenol present in the essential oils of plants belonging to the Lamiaceae family. Thymol presents a wide bioactivity; however, its use as an

antimicrobial agent is particularly interesting, as it has been shown to possess antimicrobial properties against several bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella thymimurium*, and *Pseudomonas aeruginosa*, among others. Despite the numerous uses of thymol, little is known about its potential impact on the environment, especially on freshwater microbial communities. Microorganisms are essential in river ecosystems, as they form the basis of food chains, and their activity as decomposers contributes to closing the carbon cycle.

The response of a freshwater microbial community from the Gállego river (Zaragoza, Spain) to the effects caused by different concentrations of thymol (0.1, 10, 100, and 1000 µg/mL) for 168 h was evaluated. EcoPlates Biolog™ containing 31 of the most common carbon sources in the environment were used. The data obtained were used to calculate the average well color development (AWCD) as an indicator of population growth and changes in the ability of the microbial community to metabolize substrates.

The microbial community exposed to thymol showed an intense decrease in growth activity compared to the control when exposed to 100 and 1000 µg/mL thymol. However, at 0.1 and 10 µg/mL there was an increased growth effect, suggesting the ability of the microbial community to metabolize this product. The same effect was observed on the ability to metabolize different substrates: while at the lowest concentrations (0.1 and 10 µg/mL), there was hardly any change in the metabolic profile (a slight increase was observed for amines/amides); at higher concentrations (100 and 1000 µg/mL), a decrease in the ability to metabolize almost all substrates, especially carbohydrates and amino acids, was observed.

Results demonstrate that thymol can produce changes in the growth and metabolic profile of river bacteria at concentrations around 0.1 µg/mL, although very high doses of 100 µg/mL are necessary to produce a decrease in microbial activity. Therefore, this product is unlikely to pose an ecotoxicity risk to the environment and is a good alternative to more aggressive antimicrobial products.

#### **2.12.P-Tu101 Impact of the Monoterpene Eugenol on the Physiological Profile of River Microbiota**

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Eugenol is a terpene, found in many plants and their extracts, used in perfumes, excipients, and in current odontology procedures. Thanks to the antiseptics properties of eugenol, an emerging application as antimicrobial compound in combination with commercial antibiotics is raised. Because of this, an environmental toxicity assessment is desirable prior to a more extensive use of this chemical substance.

For this purpose, we evaluated the ecotoxicity of eugenol (0.1, 10, 100, and 1000 µg/mL) on freshwater microbial communities from the Gállego River in Zaragoza, Spain. Biolog EcoPlates® were used to calculate the Average Well Colour Development (AWCD) as an indicator of the entire capacity for degrading 31 of the most common carbon sources found in natural ecosystems.

Results showed a progressive slight decrease in AWCD and metabolite consumption ability for lower concentrations (0.1, 10, and even 100 µg/mL), followed by a complete growth inhibition for the highest dose (1000 µg/mL). Namely, a decrease in the ability to metabolize carbon sources was observed, finding that polymers and carbohydrates were the most affected.

Therefore, this study concludes that, due to the wide range of properties this product offers, its use will increase in the future, and therefore, will land on the environment more often. This assessment allows us to better understand eugenol and how it would impact on the structure of river communities and modify their physiological profile before it happens.

#### **2.12.P-Tu102 Screening Toxicity of Relevant Environmental Mixtures using *Caenorhabditis elegans***

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Chemical substances from different sources enter wastewater treatment plants and end up in the environment. Due to effluent and biosolids discharges, pesticides and pharmaceuticals can be found in effluents and therefore in waterbodies or soils. This constitutes a challenging problem for the environment and human health as they occur as complex mixtures (CM) rather than as individual substances. Understanding the toxic effects of such CM remains crucial for proper risk assessment (RA) and public health safety, which are usually carried out separately. From an environmental toxicology perspective, *Caenorhabditis elegans* can be a good model organism for toxicity screening of different CM, for the environment and human health assessment.

In this study, we evaluate the single and joint toxicity of three substances, widely found in Portuguese wastewater effluents, on the survival, feeding, and reproduction rates of *C. elegans*. The chemical substances chosen were the insecticide cypermethrin, the herbicide MCPA, and the over-the-counter pharmaceutical diclofenac, as little is known about the interaction of these chemicals. Concentration addition and Independent Action predictive models were also used to understand the CM's toxicity pattern better. Interactions for synergism or antagonisms were evaluated, looking at deviations from these models.

The results obtained from our study support the importance of *C. elegans* as a model organism to be used jointly for human health and environmental hazard assessment. The fast testing of different endpoints provided different outputs (additivity, synergism, or antagonism) and sensitivities and will unravel the potential MoA of substances or their interactions in CMs, ultimately leading to the development of an adverse outcome pathway.

## 2.12.P-Tu103 When a Psychiatric Drug Alters the Migratory Behavior of the Critically Endangered Fish *Anguilla anguilla*

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In the Anthropocene, the current biodiversity losses caused by human activities have various features, from individuals to populations, species, and ecosystems. Chemical pollution is one of the major current threats, especially because Wastewater Treatment Plants do not reduce or treat psychiatric drugs leading to the chemical pollution of aquatic environments. Most of the studies in ecotoxicology focus on the effects on survival or development, losing sight of the potential effects on biodiversity. Individual behavior and the related intraspecific diversity are the main components of it, even though overlooked.

Here, we assessed the effects of a psychiatric drug on the European glass eels' migratory behavior. Glass eels are eel juveniles migrating from the sea towards rivers crossing estuaries and WWTP effluents to colonize hydro-systems. Usually, individuals colonize and distribute themselves from estuaries to upstream river systems leading to changes in local density, which drive sex-ratio and individual growth.

Since we used an anxiolytic, we hypothesized that contaminated individuals would be bolder and less active than control individuals. We put 40 control individuals and 40 individuals contaminated with diazepam at an environmentally relevant concentration through 7-day chronic contamination in a behavioral monitoring tank that mimics a river. This tank is like an annular tank enabling individuals to swim continuously either against the current (i.e., upstream movement) or with the current (downstream movement) and us to study migratory behavior in experimental facilities. We video-recorded around 1-min sequences over 7 days since the depuration of diazepam by glass eels takes 7 days. We also video-recorded the first 15 minutes since individuals hide directly under the gravel at the tank bottom as soon as we placed them in the tank. The time it took them to get out of their shelter is a proxy for their boldness.

We found the anxiolytic contamination led individuals to be bolder but less active than control individuals. The contamination altered their migratory behavior since they swam less at counter-current than control individuals. Finally, the behavioral alterations became more pronounced with time despite the depuration. The behavioral alteration we found may affect the local density and ultimately population processes, but also the local environment since glass eels are predated by several fishes and birds.

## 2.12.P-Tu104 3,4-Methylenedioxypropylvalerone (MDPV) Sublethal Ecotoxicity Assay on the Microcrustacean *Daphnia magna*

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The consumption of psychoactive substances (PAS) has increased in the last years. Among them, synthetic cathinones (SCAT) emerged as a popular group of PAS for recreational purposes. After consumption SCAT enter the sewage systems and reaches the wastewater treatment plants, which are not prepared for their removal, leading to their frequent detection in effluents and aquatic ecosystems. SCAT are designed to change nervous system function, posing unpredictable adverse effects on nontarget organisms. Considering the scarce information available on SCAT ecotoxicity, including 3,4-methylenedioxypropylvalerone (MDPV), it is urgent to assess their toxicity on exposed aquatic organisms. This work aimed to study the ecotoxicity of MDPV using as a model organism the microcrustacean *Daphnia magna*. For that, a sublethal assay using daphniids was carried out at a concentration range of 0.1 to 10 µg/L for 8 days and morphophysiological, behavioral, reproductive, and biochemical effects were evaluated. Results showed a significant increase in the heart size, area, and rate in juveniles at all concentrations tested (day 3). However, on day 8, a significant increase in heart rate was still observed (1 and 10 µg/L) but different responses were observed for heart size and area that showed a significant decrease in adults at the three concentrations studied. MDPV also affected swimming behaviour. Indeed, a significant increase in total distance and active time were observed at 0.1 and 10 µg/L and 10 µg/L, respectively, whereas a significant decrease in swimming speed was noted at 10 µg/L. No changes in reproductive and biochemical parameters were found at the selected concentrations. The present study showed the toxicity of MDPV at sublethal concentrations on morphophysiological and behavioral endpoints. Further studies are ongoing to extend the current knowledge about MDPV toxicity effects on this aquatic organism, concerning its enantiomers.

## 2.12.P-Tu105 Enantioselectivity in Toxicity of Smphetamine, 3,4-Methylenedioxymethamphetamine and Methylenedioxypropylvalerone on Freshwater Organisms (*Daphnia magna* and *Danio rerio*)

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The consumption of amphetamines and synthetic cathinones resulted in their widespread occurrence in the environment, raising concern about possible hazardous to nontarget organisms. Their metabolism in humans and biodegradation in wastewaters is stereoselective. Understanding enantiomer-specific toxicity is crucial for risk assessment of chiral contaminants. This study aimed



to investigate the potential enantioselective effects of amphetamine (AMP), 3,4-methylenedioxymethamphetamine (MDMA), and methylenedioxypropylvalerone (MDPV) in *Daphnia magna* and *Danio rerio*.

Daphnids were exposed to three concentrations for the racemate, 0.1, 1.0, and 10.0 µg/L, and two concentrations for the enantiomers (0.1 and 1.0 µg/L) for 8 days and morphophysiological, swimming behaviour, reproductive, and biochemical variables evaluated. For zebrafish assay, fertilized eggs were exposed for 96 h to racemate and enantiomers ranging from 0.02 to 200 µg/L. Embryonic development, malformations, and larvae swimming behaviour were evaluated.

(*R,S*)-AMP and (*R,S*)-MDMA and their enantiomers affected morphophysiological parameters and enantioselective effects were observed even at the lower concentrations. Both substances caused changes in swimming behaviour, but different responses were observed among racemates and enantiomers. No changes in first reproductive events were observed for MDMA whereas AMP racemate stimulated reproduction. (*R,S*)-MDPV caused an increase in heart rate and changes in swimming behaviour.

An increase in the percentage of malformations in zebrafish exposed to (*S*)-MDMA. Changes and enantioselective effects were observed in swimming behaviour. Regarding AMP, an increase in oedema area was observed in organisms exposed to (*R*)-AMP. An increase in larvae size and tail curvature was noted for the racemate. Larvae exposed to (*R*)-AMP showed a higher percentage of malformations and enantioselective effects were observed for swimming behaviour.

Exposure to these substances at environmental reported levels showed to interfere with swimming behaviour, morphophysiological, reproductive, and biochemical parameters at different developed stages of *Daphnia* and during embryonic development of zebrafish. Enantioselective responses were observed for some parameters.

## 2.12.P-Tu106 Morphometric Changes in Larval Zebrafish Exposed to Butylone

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New psychoactive substances (NPS) emerge worldwide and their consumption among young people has been increasing. Human urinary excretion of NPS and its metabolites contribute to their presence in wastewater treatment plants and water bodies. So, their presence in aquatic environments is expected, raising concern about the potential negative effects on nontarget species, like ichthyofauna. Butylone (BTL) is a synthetic cathinone that acts as an entactogen, with psychedelic and stimulant effects in humans, but no data are available concerning potential hazards to fish. Zebrafish (*Danio rerio*) is an animal model widely used in different areas of toxicology research and emerged as a popular model for investigating the potential toxic effects of NPS during early-life stages. Thus, this work aimed to evaluate the potential morphometric effects of BTL in zebrafish larvae.

For that, 10 embryos with approximately 3 hours postfertilization (hpf) were exposed for 96 hpf to different concentrations of BUT (0.01, 0.1, 1, 10, and 100 µg/L). Three replicates were performed for each concentration. After the exposure period, morphometric measurements (larva size, eye area, yolk area, oedema area, head area, and tail curvature) and malformation evaluations were assessed in larvae. Concentrations were selected to include environmental reported levels and higher concentrations to get insights about BTL toxicity.

Overall, a decrease in the larvae size (0.1, 1, and 10 µg/L), eye area (10 µg/L), and head area (0.01, 0.1, 1, 10, and 100 µg/L) was observed in comparison with the control group. Contrarily, we observed an increase in tail curvature (0.1 and 1 µg/L) and malformations percentage (like deformations in the tail, yolk sac oedema, and pericardium oedema) for 0.1, 1, 10, and 100 µg/L BTL concentrations.

These results show that BTL can interfere with morphometric parameters during embryonic zebrafish development even at environmental levels. Teratogenic effects in organisms' development can affect fish swimming and even their survival. Additional studies are being done to confirm these effects of BTL in fish.

## 2.12.P-Tu107 Effects of Methamphetamine Exposure on the Zebrafish Embryonic Development

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Illicit drugs are a global concern with significant direct and indirect adverse impacts on human health and wildlife. Among them, methamphetamine (METH) is a psychostimulant that is widely used as a recreational drug. Increased worldwide consumption and incomplete metabolism in the human body have led to the appearance of METH in the aquatic environment mainly via wastewater effluent discharge, with potential effects in nontarget organisms. Early development stages of aquatic organisms, like fish, are prone to adverse effects caused by toxins. Hence, zebrafish (*Danio rerio*) presenting semitransparent embryo has been increasingly used as a key model to study developmental biology and to assess the toxic effects of psychoactive substances during the early stages of embryonic development. Therefore, the goal of this work was to evaluate METH effects on the embryonic development of *D. rerio*.

Zebrafish embryos with approximately 3 hours postfertilization (hpf) were randomly exposed for 96 h to different concentrations of METH (0.05, 0.5, 5, 25, and 50 µg/L) and the effects on embryonic development parameters were evaluated, namely cumulative mortality (7, 24, 48, 72, and 96 hpf), spontaneous movements (24 hpf), heart beats (48 hpf), and hatching rate (72 hpf). For each concentration, three replicates were performed and a control group. Preliminary data showed that METH exposure has no effects during embryonic development, for spontaneous movements and hatching rate of zebrafish embryos. However, a slight increase in cumulative mortality and a decrease in heartbeats were observed at different concentrations of METH.

Our data indicated that METH does not affect the studied parameters of embryonic development; however, it can reduce the heart rate and increase mortality. These experiments provide a baseline for the study of the toxicity of METH during the zebrafish's early life stages. Nevertheless, additional studies are required about METH to better assess the potential risk for fish namely in the first stages of fish embryonic development because these changes can affect greatly their survival.

#### **A2.12.P-Tu108 A Class of their Own? Water-Soluble Polymer Pollution Impacting a Freshwater Host-Pathogen System**

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Microplastic pollution is a major ecological threat for aquatic ecosystems. A primary route of freshwater microplastic pollution is wastewater, where wastewater treatment plants (WWTP) fail to effectively remove microplastics (MPs) before effluent reaches river systems. This has fuelled the debate on whether 'intentionally added MPs' or primary MPs should be banned under UK/EU REACH chemical regulation and restriction. Primary MPs, often added to personal care products (PCPs), are beginning to see restrictions on their uses, but water-soluble polymers (WSP) have so far slipped through the net for global chemical regulation. Despite the widespread occurrence of WSP in the cosmetic and food industries, there are no regulations for their use. In addition to their prevalence in PCPs, the predominant use of WSP is attributed to wastewater treatment itself, where they are applied directly to aid removal of contaminants. As a result, reports of WWTP effluents with high WSP concentrations (up to 7 mg/L) are emerging, yet the environmental implications of these chemicals are poorly understood. There is new evidence of the sublethal effects of WSP, where physiological changes have been observed for freshwater invertebrates and neonate zebrafish, implying WSP may be inducing stress. Investigating the interaction of these chemicals with other biological stressors over longer exposures is imperative to determine the potential risk of WSP for freshwater ecosystems.

Utilising a host-pathogen model system, this study assesses the impact of two mass produced WSPs on fish welfare, specifically host growth, metabolic rate, and disease susceptibility, thereby defining the interaction with a second stressor (i.e., the pathogen). Analytical methods such as matrix-assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) will be applied to further examine the fate of these chemicals *in vivo*, achieving an interdisciplinary investigation into their effects on this freshwater ecological model. This is the first study of its kind investigating the chronic impacts of this neglected polymer class on a freshwater vertebrate. If negative effects of these polymers are encountered individually or in a multistressor scenario with disease, we hope that this research would encourage the consideration of WSP under UK/EU REACH, and increase awareness of these emerging contaminants globally.

#### **2.12.P-Tu109 Ecotoxicological Characterization of Wastewaters from Different Origins Obtained After Using the Chemical Precipitation Technique**

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With the increase in human population, more waste has been generated, leading to several environmental concerns. Consequently, more than ever, for a sustainable planet, it is urgent to transform waste into valuable by-products by treating, reusing, or recycling it. This is one of the aims of the project NETA-“New Strategies for WasteWater Treatment” (Portugal2020): to valorize different wastewaters (WW) by transforming them into valuable resources, namely hydric and nutrient resources.

Different methods have been developed to treat wastewater, usually combining biological, chemical, and physical processes (e.g., wastewater treatment plants). One alternative method being successfully explored is the chemical precipitation technique (CPT). In CPT, one reagent (Ca(OH)<sub>2</sub>) is added to the WW, that by ionic precipitation, will originate treated water and the formation of a precipitate (i.e., a sludge-like product). This method might be considered effective for WW treatment due to its low cost, quickness, and ease of use on a large scale. Considering the need for effluent and sludge environmental discharges, it is important to understand the efficacy of this technique as WW treatment, looking at their physicochemical characteristics, jointly with their ecotoxicological profile.

Thus, in the present study, the aim was to evaluate CPT's efficacy in treating WWs from different origins – industrial/domestic/urban – from an ecotoxicological perspective. As case studies, different WWs were used: 1) olive mill WW,

2) cheese whey WW, and 3) WW from multiple sources (receiving both domestic, slaughterhouse, and livestock WW). For all these WWs, ecotoxicological assays were conducted for both untreated and CPT-treated WW. Acute immobilization was assessed for the crustacean *Daphnia magna*, and hatching rate, survival, and malformations for the zebrafish *Danio rerio*. The results showed that toxicity was reduced for both species after CPT treatment, compared with untreated WW, overall displaying high survival rates to all tested concentrations, except in the cheese whey wastewater (with mortality at 75% and 100% of WW for *D. magna*).

These results confirm the efficacy of using the CPT technique in (some types of) WWs from an ecotoxicologically perspective. Results also highlight that the source of WW will define the quality and toxicity of the originated CPT-treated WW, and that chemical analysis by themselves are not sufficient to define the safety and disposal of WWs.

#### **2.12.P-Tu110 Context Dependent Behavioral Responses of Arabian Killifish to Sertraline and Predator Alarm Cues**

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The release of pharmaceuticals from waste treatment plants poses a major threat to aquatic ecosystems downstream of receiving waters. The antidepressant sertraline has been detected in surface waters at concentrations up to 80 ng/L and can also accumulate in sediments and aquatic organisms. Several studies show that sertraline can affect the behavior of fish at concentrations as low as 5 ng/L. Neuroactive pharmaceuticals such as sertraline can alter fish behavior leading to maladaptive responses and increasing the risk of succumbing to predators. The aim of the present study was to measure the effects of environmentally relevant concentrations of sertraline on the swimming, foraging and mating behavior of Arabian killifish (*Aphanius dispar*) in the presence and absence of alarm cues (kairomones) from predatory dragonfly larvae.

Groups of 12 male *A. dispar* were exposed for 60 days to two different concentrations of sertraline (5 ng/L, 50 ng/L, or a freshwater control), in glass aquariums (40 × 30 × 30 cm) holding 20 L of water. There were four replicates of each treatment. The behavior of the fish was measured in three experiments: (1) Shoaling behavior. The swimming behavior of groups of one, three or five fish was recorded before and after the addition of a dragonfly alarm chemical. (2) Exploratory behavior: The swimming and foraging behavior of three killifish was recorded in a complex arena (80 × 80 cm) containing three different habitats, before and after the addition of a dragonfly alarm chemical. (3). Attraction to female killifish.

Overall responses to sertraline exposure and predator alarm were strongest when single fish were measured, suggesting that solitary fish assessed the threats as greatest when they were alone compared to being in shoal. In the habitat preference test fish preferred the vegetation, and this preference increased following addition of alarm chemical. Sertraline did not affect female preference.

#### **2.12.P-Tu111 Evaluation of the Effects of Metoprolol on Microalga *Monoraphidium pusillum* and Fish *Poecilia* sp.**

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Metoprolol drug inhibits the stimulating effect of catecholamines on the heart, being the priority drug for consumption by the population in Mexico to treat hypertension. In wastewater in Mexico, the presence of metoprolol in concentrations of 1530 ng L<sup>-1</sup> has been identified. Due to the fact that the consequences of Metoprolol in aquatic organisms are not completely known, in this study an evaluation of the effects of Metoprolol in the microalgae *Monoraphidium pusillum* in their growth, its content of pigments and macromolecules and in the fish *Poecilia* sp. its oxidant, neurotoxic effects, and alterations in the macromolecule content were determined. A bioassay was carried out where the microalgae were exposed to five concentrations of the drug (20, 40, 60, 80, 100 mg L<sup>-1</sup>) for 72 h, to determine the EC<sub>50</sub> (Probit). Subsequently, it was carried out a sublethal bioassay with microalgae to evaluate Chlorophyll, Carotene, and macromolecule content. The fish were exposed to five concentrations of the drug (20, 40, 60, 80, 100, 250 mg L<sup>-1</sup>) for 96 h, to determine the LC<sub>50</sub> (Probit). Afterwards, a sublethal bioassay was performed to evaluate the degree of lipid peroxidation, the activity of the enzyme Acetylcholinesterase, and the concentrations of macromolecules. The EC<sub>50</sub> values (72 h) in the microalgae and for the fish LC<sub>50</sub> (48 h and 72 h) are in the limit of EC<sub>50</sub> 10 mg L<sup>-1</sup> ≤ 100 mg L<sup>-1</sup> indicating that Metoprolol is a harmful compound for these organisms (GHS, ONU 2011). In the tests with microalgae, an increase of between 5% to 43% in the concentration of pigments and from 27% in macromolecules were observed. In the fish the Metoprolol had an oxidative effect, AChE inhibition, proteins, and carbohydrates decreased with increasing drug concentration. Metoprolol had greater harmful effects on the fish *Poecilia* sp. exposed to sublethal concentrations.

#### **2.12.P-Tu112 Toxicity of Three Types of Surfactants in Aquatic Organisms of Different Trophic Levels**

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Surfactants are synthetic compounds used in the manufacture of detergents; generally anionic surfactants are the most used, but the use of cationic and noncationic surfactants is increasing, because they are present in personal care products. These compounds are toxic to aquatic organisms, since they destabilize cell membranes, affecting survival, and they also alter essential functions such as respiration, feeding, and growth. In this study, an evaluation of the toxicity of 3 surfactants was carried out: Lauryl Alkylsulfonate (anionic LAS), Cetyltrimethyl Ammonium Chloride (cationic), and Triton X (nonpolar) in aquatic organisms of

different trophic levels (microalgae, cladocerans, and fish), to determine which species is more sensitive to these compounds. The microalgae *Monoraphidium pusillum*, and *Pseudokirchneriella subcapitata*, six species of cladocerans: *Chydorus* sp., *Ceriodaphnia dubia*, *Daphnia exilis*, *Daphnia magna*, *Moina macrocopa* and *Simocephalus mixtus* and two species of fish *Danio rerio* and *Poecilia* sp. were exposed to 5 concentrations of surfactants (for 48 hours for cladocerans and 96 hours for tests with microalgae and fish). With the mortality data obtained, the Lethal Concentration 50 (LC<sub>50</sub>) was determined (Probit method). The sensitivity of the species was determined by comparing their LC<sub>50</sub> values. The LC<sub>50</sub> values obtained in the tests with LAS varied from 0.04 to 110 mg L<sup>-1</sup>, with Cetyltrimethyl Ammonium Chloride from 0.032 to 2.99 mg L<sup>-1</sup>, and with TX from 4.3 to 48.029 mg L<sup>-1</sup>. The most toxic surfactant for the 10 species was the cationic surfactant and the least toxic the nonpolar. Cladocerans were the most sensitive organisms to surfactants. The species most sensitive to surfactants were *Pseudokirchneriella subcapitata* and *Daphnia exilis*. The degree of toxicity of these compounds ranged from highly toxic to harmful, according to the Globally Harmonized System (GHS).

## 2.12.P-Tu113 Drinking Water Production – What Are the Risks From Dumping RO-Concentrates and Antiscalants into the Aquatic Environment?

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To ensure high drinking water quality, modern techniques like membrane-filter like reverse osmosis (RO) technologies are constantly applied and optimized. During the water purification process, concentrates that contain highly concentrated salts, RO-additives, and partly micropollutants are produced. Subsequently, these concentrates are discharged either directly or indirectly into surface water. The potential impact of these discharges has not yet been fully investigated. The RO-additives and antiscalants (AS) are needed to elongate the membrane lifetime. Once leaked into the environment, their high complexation ability could lead to sediment sealing and enhance the migration of heavy metals. Also, AS shows a low biodegradability and could aggravate phosphate, contributing to eutrophication. In the *KonTriSol* project, various AS active substances, AS technical products, and RO-concentrates were tested to be supplied with a more precise statement on the effect of the RO-concentrate discharge on the environment. The *KonTriSol* project aims to develop a comprehensive bioassay battery for the ecotoxicological assessment of AS and concentrates, evaluate the effects of AS active ingredients in complex mixtures and concentrate treatment strategies. Additionally, concentrate discharge conditions should be optimized. For a comprehensive ecotoxicological evaluation, AS active ingredients, AS technical products, RO-concentrates, and treatment strategies were tested in a biotest battery, with acute (on daphnids, fish and algae) and mechanism-specific endpoints (genotoxic-, PFAS-like-, endocrine-activity). We found that AS and its technical products have no endocrine or genotoxic effects but exhibit acute toxic effects toward daphnids, especially algae. As there are toxic effects already present in potentially environmentally relevant concentrations, AS discharge could have a potential harm on the environment. The first results show that RO-concentrates containing AS can exert an (low) acute toxic effect on daphnids and fish (algae toxicity data yet to come). It must be considered that the concentrates are diluted when discharged into the environment, which will lower the effects. With the knowledge about the ecotoxicological properties of RO-concentrates and AS, concentrate discharge conditions into the environment can be optimized, thus preventing damage to the aquatic environment.

## 2.12.P-Tu114 Ecotoxicity of Combined Exposure of Antibiotics and Biofilms to *Xenopus laevis* Larvae

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Over 40 different antibiotics are found in wastewater treatment plant effluents (WWTP) and freshwater (FW) ecosystems, usually in mixture. They are rejected in environment through different sources at low concentration. Organisms in freshwater such as wild animals and biofilms (multicellular community of microorganisms) are constantly exposed to antibiotics. To understand ecotoxicology of antibiotic cocktails in environment, this experiment has been conducted to assess combined effects from antibiotic cocktails and various biofilms with different exposure scenarios in more environmental relevant conditions. In accordance with international standard ISO 21427-1 Guidelines, *Xenopus laevis* larvae have been exposed 12 days to 10 µg.L<sup>-1</sup> to cocktail 1 (six antibiotics) or cocktail 2 (three antibiotics) reported on EU Watchlist (ciprofloxacin, azithromycin, amoxicillin, erythromycin, trimethoprim, sulfamethoxazole), and with or without biofilm (from WWTP outlet, or from FW). Morphometric parameters (length, growth rate), genotoxicity (micronucleus induction), and oxidative stress biomarkers data have been collected in larvae exposed in different conditions compared to control. After 12 days, morphometric parameters decreased for larvae exposed to antibiotic cocktail 1 + biofilms present (WWTP or FW). Genotoxicity has been reported in larvae exposed to biofilm (WWTP or FW) and with cocktail 2 + WWTP biofilm. Based on current data from this study, hypotheses can be drawn on ecotoxicity of biofilms but need further research. Oxidative stress biomarkers analysis is currently ongoing. Investigations of potential link between gut microbial communities and genotoxicity would be explored by further analysis of microbial diversity in gut larvae and biofilms.

## 2.12.P-Tu115 The Challenges of the Risk Assessment of Textile Wastewater

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During textile wet-processing, which includes garment dyeing, thousands of chemicals are used with different toxic potentials and modes of action. Alone the used textile dyes contain several toxic and stable compounds. If these are released into the water system, by discharging untreated or inadequately treated wastewater into rivers, this has serious implications for human and

environmental health, as can currently be observed in major producing countries such as India. However, the impacts of industrial wastewater on organisms and the environment are difficult to assess because of the complex composition and mixture of chemicals, including potentially degraded products, which might be more toxic than the parent compounds. In addition, the high salt concentration in textile wastewater can lead to the salinization of rivers, which could negatively impact freshwater organisms and change the toxic potential of the substances. Thus a realistic risk assessment requires the use of holistic and impact-oriented methods. Chemical analysis cannot fully detect the complexity of substances, so in this study, the wastewater samples from a textile dyeing plant in India were analysed both chemically and ecotoxicologically. Based on the data from the chemical target screening using liquid chromatography–high-resolution mass spectrometry (LC-HRMS, compounds belonging to three main classes could be found: substances from textile wet production, pesticides, and pharmaceuticals. In addition to the analyses of the textile effluents, the toxic potential of five reactive azo dyes used in the dye factory was also investigated using various endpoints. The toxic potential varied widely among these dyes. For example, the acute toxicity in zebrafish larvae after 120 hpf exposure was 4.5 times higher for the reactive azo dye ME3B red compared to yellow GD3R. As described, the high salt concentration in textile effluents is also an additional stressor, especially for freshwater organisms. Toxicity studies with and without the addition of salts showed an increase in toxicity in different organisms in the mixture compared to exposure to salts or dyes alone.

## 2.12.P-Tu116 Integration of Effect-Based Methods in the Development of Textile Wastewater Technologies

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Only 3% of the water on earth is freshwater and therefore a limited resource. In addition, factors such as the growing population or pollution from agriculture and industrial wastewater are leading to an increased scarcity of freshwater resources. Due to its high population density and increasing industrialization, India is particularly affected by the consequences of freshwater scarcity. The textile industry is one of India's most important economic sectors and is one of the main producers of industrial wastewater worldwide. In addition, textile wastewater is particularly difficult to treat due to the high number of different chemicals (e.g., reactive azo dyes) and high salt concentration. Therefore, there is a need to develop wastewater treatment technologies that meet the requirements of treating complex textile wastewater. The EfectroH<sub>2</sub>O project is developing a new wastewater treatment technology that combines advanced oxidation processes (AOP) for the removal of micropollutants and capacitive deionization (CDI) for selective salt removal and recovery. The development will be accompanied by effect-based methods, as chemical analysis alone cannot detect the toxic potential of mixture effects or metabolites to investigate the efficiency of the new technology. For this purpose, a synthetic textile wastewater is produced in the laboratory based on data from the wastewater of the textile industry MS/Rohini, Erode in Tamil Nadu, India. The synthetic wastewater contains the five dyes Red GDN, Navy GDG, Red ME3B, Yellow GD3R, Black GDNN [2.9 g/L] and a salt mixture [7 g/L] of NaHCO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, NaCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub>. The toxic potential of the synthetic textile wastewater will be tested on a laboratory scale before and after treatment with the newly developed technology. The acute toxicity is analyzed using freshwater model organisms in different trophic levels (Algae, Daphnids, and Fish embryo). The first results show that *Daphnia magna* has a lower EC<sub>50</sub> value (2.03 g/L saltmix and 0.75 g/L dyemix) than *Danio rerio* (2.34 g/L and 0.88 g/L). The main sublethal effects in *D. rerio* were fin deformations, edema, and prevention of hatching. Moreover, the data for the acute toxicity with algae *Raphidocelis subcapitata* and specific endpoints for genotoxicity will be available at the conference.

## 2.12.P-Tu117 Evolution of the Endocrine-Disrupting Activity of BPA and Analogues During Aqueous Ozone- and •OH-Based Oxidation

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As a result of their proven, endocrine-disrupting effects, bisphenol A (BPA) is currently a restricted chemical in different world regions. The immediate solution for companies using this compound in their manufacturing processes has been its replacement by other bisphenol analogues, such as BPF, BPS, BPAF, and BPC, among others. However, several previous studies have revealed endocrine-disrupting effects of these substitutes over organisms similar to those exerted by BPA. In this research, the endocrine-disrupting activity of BPA and some of its mostly employed substitutes was studied before and after both ozone and hydroxyl radical oxidation-based treatments of aqueous solutions containing these chemicals. The gene expression responses of the model organism zebrafish have been used for toxicological evaluation.

The selected compounds were BPA, as well as the analogues BPS, BPAF, and BPC-Cl. Zebrafish eggs were obtained by natural mating of adult individuals, which were collected at approximately 17 h postfertilization (hpf), then rinsed and finally checked for fertilization. Embryos were placed in six multiwell plates and their exposure to several concentrations of selected bisphenols was conducted from 4 to 5 days postfertilization (dpf). After RNA extraction, the endocrine disrupting effects were evaluated by comparing the expression of different genes of the control well (i.e., not containing bisphenols) with the different concentrations that were selected.

Results revealed that the gene displaying the highest expression was the estrogenic gene *cyp19a1b*. The concentrations evaluated were 0.5, 2.5, 5, 25, and 50 mM. In the case of BPA, to cite an example, the highest expression of gene *cyp19a1b* compared to the control was observed at a concentration of 25 mM. The lower expression observed after exposure to concentrations of 50 mM was attributed to an increase of nonspecific toxicity, according to the observed decrease of the swim bladder inflation rates.

The evaluation of toxicity evolution over ozone- and •OH-based treatments, performed by means of these techniques, gives further insights into treatment characterization, providing practical knowledge to assist in the selection of the optimal treatment conditions for these water treatment technologies.

#### **2.12.P-Tu118 Detoxification of Wastewater Plant Effluents by Soil Aquifer Treatment Methodologies**

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Despite all the efforts carried out in Wastewater Treatment Plants (WWTP), their effluents contain a number of chemicals, many of them classified as contaminants of emerging concern, such as pharmaceuticals and personal care products, steroid hormones, surfactants, industrial chemicals, and pesticides. Many of these chemicals are well known to have endocrine disrupting properties (such as estrogenicity) and dioxin-like activity which are capable to alter the general development, metabolism and endocrine system of organisms living downstream. In the present study, we have investigated the efficiency of three mesoscaled Soil Aquifer Treatment (SAT) systems equipped with reactive barriers (RB), containing different proportions of sand and organic fillings and associated to a sandy aquifer, to reduce toxicological risks. SATs were fed with secondary effluents from an operating WWTP during two consecutive campaigns timed before and after the 2020 summer vacation period. Fifteen water samples were collected from the WWTP effluent, after every RB, and at the end of the aquifer. Transcriptomic analyses of zebrafish embryos exposed to the corresponding extracts revealed a wide range of toxic activities in the WWTP secondary effluents. Our results demonstrated that the associated responses were reduced by more than 70% by SAT, achieving control levels in some cases. Similar results were obtained when human HepG2 hepatic cells were tested for cytotoxic and dioxin-like responses. Toxicity reduction appeared to be partially determined by RB composition and/or SAT managing, and it correlated with the removal of certain CECs by SAT, as monitored by nontarget semiquantitative LC-HRMS analysis. In conclusion, SAT appears to be a very promising technical approach for efficiently reducing the effects of recalcitrant pollutants from WWTP secondary effluents on the environment and human health

#### **2.13.P New Approaches and Methodologies for the Future Direction of Pollinator Health and Safety**

##### **2.13.P-Mo133 Tissue Specific Investigation of the Detox Gene Inventory of *Apis mellifera* After Exposure to Coumaphos, Malathion, and their Metabolites**

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Pollinators such as honeybees are among the most ecologically important insects because they play a major role in plant reproduction, thus contributing to the functioning of various ecosystems. In recent years high annual losses of insect pollinators were reported, which have been linked to various environmental stressors including the exposure to pesticides. Consequently, it is important to gain a deeper understanding of the detoxification mechanisms of the honeybee and thus the factors driving honeybee sensitivity toward pesticides.

In this study we focus on a range of established detoxification enzymes including the cytochrome P450s, which are known to mediate tolerance to pesticides in the honeybee. In order to identify potential genes involved in pesticide detoxification we determined the expression profiles of candidate genes suspected to be involved in pesticide detoxification in different honeybee tissues prior to, and following exposure to the pesticides coumaphos and malathion, and their metabolites coroxon and malaoxon. Next we investigated the causal involvement of these enzymes by performing acute contact toxicity tests with adult honeybees exposed to the pesticides with and without enzyme group specific inhibitors.

We found that some genes had particularly high transcript levels in excretory tissues. These include genes encoding for cytochrome P450 monooxygenases such as *CYP9Q3*, which is already known to be involved in the detoxification of neonicotinoid insecticides, and some *MRP4* genes, which encode proteins of the family of ATP-binding cassette (ABC) transporters and are known for their involvement in export of pesticides and their metabolites. Also, a single glutathione-S-transferase gene (*GstDI*) had increased transcript levels in the Malpighian tubules and the fat body of the honeybee.

In addition, some *MRP4* genes as well as genes encoding for cytochrome P450 monooxygenases, such as *CYP4C1* and *CYP4C3*, were upregulated in response to pesticide treatment, and the combination of pesticides of interest with some synergists resulted in increased mortality. In summary, this study represents a first overview of the tissue-specific distribution of expression of detox genes and their response after exposure of coumaphos, malathion, and their metabolites in the honeybee and provided mechanistic support for the causal involvement of cytochrome P450 monooxygenases, carboxylesterases, and ABC transporters in detoxification of pesticides.

##### **2.13.P-Mo134 Using Machine Learning for Trait-Based Predictions of Insecticide Toxicity to Wild Bee Species**

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Honeybees (*Apis mellifera*) are traditionally the main focus of pollinator risk assessment. However, wild bee species are often more vulnerable to stressors than honeybees, and it is unclear whether the current application of safety factors to exposure thresholds can offer them enough protection. The revised guidance on the risk assessment of plant protection products on bees

addresses this issue by incorporating some wild bee species (e.g., bumble bees of the genus *Bombus* and solitary bees like *Osmia* spp.) as standard test organisms into the requirements for the registration of plant protection products in the EU. The problem however remains the same: Even if we know how several bee species are affected by a chemical, we still do not know whether this adequately protects all other wild bee species. *In silico* techniques that enable chemical toxicity estimation for untested pollinator species based on a mix of chemical properties and bee traits are promising tools to address this task.

We present here a flexible machine learning model (Boosted regression tree) trained to predict LD50 values of insecticides to untested bee species using a combination of the chemicals' molecular fingerprints and physicochemical properties, and bee species specific traits including ecological characteristics, size measurements, taxonomic information and known detoxification genes (cytochromes P450). We show how machine learning models combined with explainable artificial intelligence (xAI) can be used to derive hypotheses on bee-chemical trait combinations leading to particularly toxic or non-toxic outcomes. We argue that this trait-based approach is a promising addition for assessing the risk of chemicals to wild bee species that cannot be tested in the lab, and will improve our mechanistic understanding of insecticide toxicity.

### 2.13.P-Mo135 Ecological Traits Interact with the Landscape Context to Determine Bees' Pesticide Risk

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Widespread contamination of ecosystems with pesticides threatens nontarget organisms. However, the extent to which life-history traits alter pesticide exposure and resulting risk in different landscape contexts remain poorly understood. We address this for bees based on pesticide concentrations in pollen and nectar collected by *Apis mellifera*, *Bombus terrestris* and *Osmia bicornis*, representing extensive, intermediate, and limited foraging trait types across a gradient of agricultural land use. We found extensive foragers (*A. mellifera*) experienced the highest overall pesticide risk – additive toxicity-weighted concentrations. However, intermediate (*B. terrestris*) and limited (*O. bicornis*) foragers' risk estimates responded to the landscape context, resulting in increased pesticide risk with more agricultural land. Furthermore, pesticide risk correlated among bee species and between pollen and nectar food sources, demonstrating the potential for proxies useful in pesticide risk assessment. Our work provides foraging trait and landscape-dependent information on the identity, level and frequency of pesticides bees encounter in agricultural landscapes. Such information is fundamental for developing a more holistic and realistic environmental risk assessment and essential for taking action to meet policy goals to reduce the pesticide-related risk for bees and other pollinators.

### 2.13.P-Mo136 Solitary Bees and Pesticide Exposures: A Model Approach

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Solitary bees, including both wild and managed populations, are important pollinators of crops and wildflower communities. Solitary bees can potentially be exposed to pesticides via multiple routes of exposure which may differ between species and between solitary bees and the Western honey bee (*Apis mellifera*) which is currently used as surrogate for risk assessments across bee species. Species-specific traits may additionally interact with the potential for exposures and effects, including, for instance, phenology, reproductive rates and flower preferences. We are presenting a population model for solitary bees in agricultural landscapes, SolBeePop. The model was developed to simulate a variety of species by using species-specific ecological traits as model parameterisations. Model parameterisations for several species (*Osmia bicornis*, *O. cornifrons*, *O. cornuta*, *O. lignaria*, *Megachile rotundata*, *Nomia melanderi*, and *Eucera (Peponapis) pruinosa*) were compiled from the literature whereby data availability varied by species. Exposures to a pesticide through multiple exposure routes can be considered, such as nectar, pollen, direct spray and nesting materials. Effects are implemented using a simplified toxicokinetic-toxicodynamic model, BeeGUTS, adapted specifically for adult bees while an exposure-response functions is applied to simulate effects to developing in-nest life stages. We calibrated and validated the model with control data from semifield studies conducted with *O. bicornis*. We applied the model across the model species to assess the impacts of different trait combinations on population dynamics as well as population-level effects. The model provides a valuable tool for higher-tier pesticide risk assessments across species of solitary bees in agricultural landscapes.

### 2.13.P-Mo137 Predicting Pollen and Pesticide Occurrences at National Scales Using Citizen Science Monitoring Data

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Honeybees (*Apis mellifera* L.) are one of the key species providing pollination for crops and a variety of other flowering plants. The quality of the environment is paramount for the well-being of honeybee colonies, as landscapes with a too low forage quality can be detrimental to colony performance. In addition, such landscapes have been linked to an increase in frequency and intensity of pesticide use, thus providing an extra stress. In order to survey landscape quality, there is a need for a general approach that allows for an assessment of likely crop diversity and the prevalence of pesticides in an area over the course of a season.

Data on pollen and pesticides were collected in five apiaries in Austria and four apiaries in Denmark using a standardised protocol. Two types of geo data were used to extract predictor variables. Firstly, sites were chosen to cover different landscapes

based on CORINE landscape classes. Secondly, the cumulative degrees Celsius above 10°C after the 1st of January in 2019 was used as a predictor variable for pollen (as a proxy for plant phenology).

Prediction of the probability of a pollen family or a pesticide being present in a location (i.e., multiclass classification) can be achieved using random forests, a machine learning technique. Each time an observation in the training data is passed through a classifier tree it is placed into a certain outcome category (predicted outcome); each tree can lead to a different outcome for the same sample. Next, from the ensemble of classifiers (i.e., the forest) an estimated probability is given for a sample being a member of a certain category, as the proportion of all the trees that predict that category for the sample (observation).

Predictive maps were created of: 1) pollen diversity, and 2) pesticide diversity. Sparse training data lowered the performance of the models. Nonetheless, the predictions seem to match with an expectation of lower numbers of pesticides in areas with a higher floral diversity. In a follow-up project, data will be collected in all 27 EU countries. Hence the models can be updated, and it is expected this will result in models that can be used to make risk maps for pollen and environmental pollutants that can be used by, for example, beekeepers to find suitable locations for their apiaries. Moreover, these maps will show, in time and space, where mitigation measures may be needed, allowing authorities and managers to take efficient and targeted action.

### **2.13.P-Mo138 *In vitro* Impacts of Acute and Chronic Exposure to $\lambda$ -Cyhalothrin and Spinetoram on the Honeybee (*Apis mellifera*) Larvae**

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$\lambda$ -Cyhalothrin is a pyrethroid insecticide that interferes with the nervous system of insects, which is effective against aphids. A recent study reported that  $\lambda$ -cyhalothrin influenced the performance and the activity of learning and memory, and reduced homing ability of honeybees. Spinetoram is a spinosyn insecticide that targets the insect nervous system of the nicotinic acetylcholine (nACh) receptors and  $\gamma$ -aminobutyric acid (GABA) gated ion channels. The honeybee exposed to spinetoram showed a reduction of sugar solution consumption compared to the control, which can potentially impact the health and growth of honeybee colonies. As there are limited studies about the potential impact of both pesticides on honeybee larvae relative to adult bees, we investigated the acute and chronic toxicities of  $\lambda$ -cyhalothrin and spinetoram on honeybee larvae reared *in vitro*. The 72 h LD50 values of  $\lambda$ -cyhalothrin and spinetoram were 0.058 (0.051-0.066) and 0.026 (0.01-0.045)  $\mu\text{g a.i./larva}$ , respectively. In the chronic toxicity test, the LD50 at D22 of  $\lambda$ -cyhalothrin and spinetoram were 0.040 (0.033-0.046) and 0.017 (0.014-0.019)  $\mu\text{g a.i./larva}$ , respectively. The chronic NOED of  $\lambda$ -cyhalothrin and spinetoram was 0.0125  $\mu\text{g a.i./larva}$ . All  $\lambda$ -cyhalothrin treatment groups had an adult deformation rate of higher than 30%, exhibiting a significant difference compared with solvent controls in a dose-dependent manner. Similarly, spinetoram treated bees exhibited significantly more deformities in comparison to the solvent control. The acute and chronic LD50 values estimated in our study suggests both pesticides are highly toxic to bee larvae. Both pesticides significantly increased the deformation rates of emerging bees. Our results would provide some important findings for the lethal and sublethal effects of these insecticides on honeybee larvae. Further in-depth understanding is needed to confirm the effect of both pesticides based on molecular mechanisms.

### **2.13.P-Mo139 Flupyradifurone: Systemic Butenolide, Persistent and Bioaccumulation in Honeybee Hives and Hummingbirds in British Columbia, Canada**

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In Canada, new regulations for the use of imidacloprid were implemented in April 2021 on high bush blueberry crops. Imidacloprid use was reduced from two sprays per year to one spray and only after bloom drop. There was no change to use of flupyradifurone, a systemic butenolide that was introduced since 2015, and it is rapidly replacing imidacloprid for aphid control. Although flupyradifurone is less acutely toxic to invertebrates it is sprayed at almost 10 times the application rate as imidacloprid. In the Fraser River valley in British Columbia blueberry crops are rapidly expanding in acreage. Flupyradifurone is now being detected at concentrations an order of magnitude above imidacloprid levels in honeybee nectar, honey, and hummingbird urine. Flupyradifurone concentrations were as high as 418 ppb in nectar, 156 ppb in honey, and 43.9 ppb in hummingbird urine among 12 study sites in a gradient from within 200 m to 22 km from blueberry crops. EU maximum residue levels acceptable in honey for flupyradifurone were also exceeded in samples of honey from hives within 200 m and up to 2km of blueberry crops.

### **2.13.P-Mo140 30 Day Acute and Chronic Honeybee Test with Microbials**

**Eugenia Soler<sup>1</sup>**, Emmanuelle Verge<sup>2</sup>, **Carmen Gimeno<sup>3</sup>** and Josep Antoni Aguilar-Alberola<sup>3</sup>, (1)EUROFINS TRIALCAMP, SLU, Alcacer, Spain, (2)Eurofins Agrosience Service Ecotox GmbH, Niefern-Öschelbronn, Germany, (3)Eurofins Trialcamp, Spain

For microbial testing, two guidelines are available, OPPTS 885.4380 and OECD 67. Both are very general and do not give enough details on test design, endpoints, or validity criteria.

Nowadays, acute (oral and contact) and chronic toxicity tests based on OECD 213, 214, and 245 are conducted for the registration of new or existing microbial products in the market. However, it is necessary to develop new methodologies to fulfil the demands of microbial testing in ecotoxicology.



We are sharing our previous findings and working hard, together, with the ICPPR Microbials and bees working group (launched at the ICPPR 2017). As a group, we are working on several approaches and methodologies to establish new guidelines in the near future.

This poster presents one approach to keep the bees alive for 30 days with less than 20% mortality. Under this approach studies can be run.

### 2.13.P-Mo141 Underestimated Adverse Effects of Entomopathogenic Nematodes to Bees

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There is a great interest in finding sustainable alternative plant protection products to safeguard biodiversity and our ecosystem. Entomopathogenic nematodes (EPNs) have received much attention as an alternative biological-control agents to conventional synthetic agrochemicals. However, as nematodes are considered natural enemies, national authorities often approve commercial products based on limited or no data. Here we show for the first time that foliar exposure to a commercial EPN product can significantly reduce honeybee survival and that the nematodes can successfully replicate within the carcasses of adult bees. Following an adjusted EPA protocol for assessing the toxicity of chemical residues on foliage, we exposed newly emerged adult honeybees (*Apis mellifera*) and larvae of the greater wax moth (*Galleria mellonella*) for up to 96 h to a low (0.25 Mio/m<sup>2</sup>) and medium (0.5 Mio/m<sup>2</sup>) field realistic concentration of *Steinernema carpocapsae*. Daily mortality was recorded over four days and nematode reproduction was assessed in all dead individuals. The data revealed that, *S. carpocapsae* exposure significantly reduced wax moth larvae survival (both *p*'s < 0.001), resulting in an increased mortality rate of 80% across both treatment groups compared to controls. Likewise, both the low and high concentrations of *S. carpocapsae* significantly reduced honeybee survival (both *p*'s < 0.001). However, the data revealed a dose-dependent effect wherein the high exposure led to a significantly higher mortality rate (55%) than the low (43%) when compared to the controls. Nematode reproduction was significantly higher in the wax moths compared to the honeybees (*p*<0.001), yet no significant difference was observed between the low and high treatment groups for either species (*p*>0.56). Average nematode reproduction per individual wax moth and honeybee was 126 695 and 4370, respectively, representing a 29-fold increase in the wax moth compared to honeybees. The data provide clear evidence that *S. carpocapsae* exposure can impose negative lethal effects as well as reproduce in honeybees. Considering the vast lack of data regarding potential adverse effects of EPNs on nontarget pollinating insects, our results underline the urgent need to act cautiously when considering foliar application of EPNs on crops. Further research is required to adequately address the potential risk of EPNs to bees and other NTA's pollinating insect species regarding foliar and soil application.

### 2.13.PC New Approaches and Methodologies for the Future Direction of Pollinator Health and Safety

### 2.14 New Developments in Sediment Ecotoxicology and Risk Assessment

#### 2.14.T-01 Effect of Chronic Exposure of Sediment-Associated Neurotoxic Pharmaceutical Sertraline on the Reproduction of Benthic Deposit Feeder, *Tubifex tubifex*

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Among the emerging contaminants, pharmaceuticals are considered one of the most pertinent substances that may pose threats to aquatic ecosystems. Pharmaceuticals are designed to be directed at specific metabolic and molecular pathways. Thus, they are assumed to still be biologically active when entering the ecosystem and may therefore result in unpremeditated impacts on nontarget organisms. One of the most widely used selective serotonin reuptake inhibitors, sertraline (antidepressant), is regularly found in aquatic environments. However, knowledge about effects of this sediment-associated pharmaceutical in benthic invertebrates is insufficient. The present study examined the impacts of chronic exposure (28d) (3.3, 33, 330 µg/g) of sediment-associated sertraline on reproduction in the deposit-feeding oligochaete, *Tubifex tubifex*. Sertraline significantly decreased growth and survival of *T. tubifex*. Worms exposed to higher sertraline concentrations had a lower reproduction rate indicated by a significantly lower number of cocoons produced. Worms exposed to environmental relevant concentrations have shown a reduction in growth in exchange for an increased reproduction rate. The presented results indicate that sertraline has negative impacts on *T. tubifex*'s survival, growth, and reproduction. However, information regarding molecular and behavioural changes leading to growth and reproduction impacts of invertebrate under chronic sertraline exposure is still inadequate; further research into chronic toxicity is therefore advocated.

#### 2.14.T-02 From the Sediment into the Biomass: Microplastic Uptake in a Protected Sediment Dwelling Species

Flora Rendell-Bhatti<sup>1</sup>, Elma Lahive<sup>2</sup>, Colin Bull<sup>3</sup>, Richard Cross<sup>2</sup>, Gbotemi Adediran<sup>2</sup> and Ruairidh Cox<sup>2</sup>, (1)Biological and Environmental Sciences, University of Stirling, United Kingdom, (2)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom, (3)University of Stirling, United Kingdom

Plastic contamination of freshwater ecosystems is widely distributed, with hotspots of increased abundance in deposited sediments. Benthic animals occupying these depositional habitats such as lamprey larvae contribute to ecosystem processes, including nutrient cycling. However, whether protected vulnerable species such as these are exposed is a pertinent question when trying to understand the consequences of plastics in the natural environment. Interactions between plastics and freshwater biota are regularly being documented, and its integration into aquatic food webs provides a route for remobilisation and trophic transfer. This study analyses microplastic loadings in paired samples of sediments and lamprey larvae to allow a direct comparison of microplastic loadings, using micro-Fourier transform infrared spectroscopy. We detect microplastics in all samples

of lamprey and paired sediment, ranging in abundance from 1.00 to 27.47 particles g<sup>-1</sup> in dry lamprey gastrointestinal tract tissue, and 0.40 to 105.41 particles g<sup>-1</sup> in dry sediment. The most encountered polymer types in lamprey from their immediate habitat were polyurethane, polyamide, and cellulose acetate. Microplastic abundance in lamprey was not correlated with that of the surrounding sediment, suggesting that either specific polymer types are retained or other factors such as larvae residence time within sediment patches may influence biological uptake. It is crucial to understand the fate and transport of microplastic contamination, thereby identify potential stressors to populations. However, this is often limited by the collection of threatened species and the analytical challenges to quantify smaller-sized plastics in complex organic matrices. We overcome these challenges to provide novel findings, which reveal microplastic particles found above the limit of detection in all samples. To the best of our knowledge, this is the first study to document microplastic contamination of lamprey larvae and surrounding sediment, contributing another potential stressor to the population status of this vulnerable species. This highlights the importance of considering microplastics in both sediment and the benthic community in integrated sediment quality assessments.

#### **2.14.T-03 Conducting Chronic Sediment Toxicity Studies to Reduce Uncertainty for Plant Protection Products**

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In Europe, a tiered approach is used to demonstrate low risk to nontarget organisms to register a plant protection product. For the aquatic risk assessment, a tiered approach is used to demonstrate low risk to organism(s) that may be exposed *via* off-field movement (i.e., spray drift, runoff, drainage). As per guidance from the European Food Safety Authority (EFSA) Aquatic Guidance Document (2013), tier 1 single species are tested, and endpoints are adjusted using assessment factors (AF) to derive regulatory acceptable concentrations (RAC). The RACs are then compared to Predicted Environmental Concentrations (PEC) in the water or sediment to derive PEC/RAC ratios. If 1 risk is not demonstrated using the results from one or two surrogate test organisms, additional testing can be conducted. This can be in the form of additional single species tests in the laboratory to derive a geometric mean (Tier 2A) or a species sensitivity distribution (Tier 2B), or mesocosm studies (Tier 3). The endpoint used in the final risk assessment uses the results from multiple single species tests as either the HCx (hazard concentration) from a Species Sensitivity Distribution (SSD) or the Geometric Mean of the No Observed Effects Concentration (NOEC) study endpoints from laboratory tests or as a No Observed Adverse Effect Concentration (NOAEC) from the mesocosm tests. A lower assessment factor may be applied because the number of diverse species tested reduces the uncertainty around interspecies sensitivity.

A higher tier risk assessment provided in this poster uses endpoints from chronic laboratory studies conducted on a wide variety of freshwater and marine aquatic benthic invertebrates exposed to sediment spiked with a plant protection product. The objective is to lower the uncertainty by increasing the number and diversity of sediment organisms tested. This approach to resolving long-term risk to sediment organisms is especially challenging considering the vastly different feeding traits, life history patterns, and life cycles for each organism tested. For this reason, there have been few if any attempts made to conduct a higher tier long-term multi-single species test program for plant protection product registration. In this poster, the methodology and results of the higher tier tests are presented. The long-term study program with eight sediment invertebrate organisms was successfully conducted resulting in a higher RAC, with a greater degree of certainty.

#### **2.14.T-05 Extending a Quantitative Weight of Evidence Approach to Include 'Omics in Sediment Quality Assessment: The Case Study of a Venice Lagoon Canal**

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In the context of ecological risk assessments, it is now well-established that ecosystem health status should be assessed based on a broad range of indicators possibly tailored to the characteristics of a specific waterbody. This holds also true in the sustainable management of dredged sediment, for which a thorough characterization is desirable so that neither the dredged area nor the destination site would result impaired.

The combination of different scientific evidence within a quantitative Weight of Evidence (QWoE) approach seemed then a valuable tool to expand the existing knowledge on the quality of bed sediment from a canal subject to future dredging in the Venice Lagoon. To this end, a series of experimental investigations were carried out. Data from the chemical characterization of inorganic and organic contaminants in one-metre sediment cores converged into a single line of evidence (LoE). Sampled sediment provided the exposure matrix for a battery of ecotoxicological bioassays, bioaccumulation tests, and biomarker analyses. The exposure and effect investigations constituted three more LoEs. Given its modularity and with the intent of reaching a better-supported risk characterization, the QWoE methodology for sediment quality assessment borrowed from Regoli et al. (2019) was extended to eco-toxicogenomics analyses. A new LoE based on the evaluation of whole-transcriptome effects of chemical exposure on bivalves was then introduced in the quantitative framework. For each of the five LoE, a set of algorithms helped in summarizing high dimensional data into synthetic hazard quotients with a relative class of hazard (from absent to severe), which are then aggregated to quantitatively infer the level of risk.

Hazard in the investigated sites was classified from slight to major, revealing a deterioration in sediment quality for sites closer to Porto Marghera industrial area. QWoE proved to be effective in summarizing a large amount of heterogeneous data, taking into account exposure to and effects of sediment at different levels of the organism functioning. Although, the integration of data into

a quantitative WoE implies a trade-off between the synthesis and the loss of information from individual LoEs. Moving backward, from the final class of hazard to the original data contributing to each LoE, is hence fundamental to correctly support risk assessment and regulatory decision-making.

## 2.14.P New Developments in Sediment Ecotoxicology and Risk Assessment

### 2.14.P-We102 Lead Sinkers in Sediments Impact Aquatic Snails

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Loss of sinkers by sports fishermen and remnants of lead shot cause a build-up of lead in aquatic environments. Although mostly sinking to the sediment, part of the lead dissolves to the water phase, and ingestion of solid lead poses a direct risk to birds. As a result, a 2021 ECHA proposal calls to phase-out lead in sport fishing and hunting. This will be decided upon in 2023. To assess the potential impact of lead in Dutch surface waters, a literature review comprising the amount of lead lost by sports fishermen, measurements of dissolved lead in surface waters, and effect concentrations was performed. This review was complemented by an experimental study to assess the impact of lead on aquatic snails.

The available data on measured lead concentrations in Dutch surface waters showed that yearly average values of freely available dissolved lead are below the Dutch water quality limit (JG-MKE) of 1.2 µg/L. Species Sensitivity Distributions in literature indicate that aquatic snails are amongst the most sensitive organisms, with reported NOEC values of 12 µg/L. In addition, a laboratory fate study with 4 pellets in 500 mL overlying water reported that at neutral pH conditions dissolved lead concentrations can exceed the JG-MKE. Consequently, we investigated the leaching potential and impact of 3 mm lead sinkers on the aquatic snail *Physella acuta*, with and without sediment. Twenty-eight days of exposure to 4, 20 or 100 lead sinkers (1 month pre-aged) did indeed exceed the JG-MKE, and sediment treatments reduced growth of the snails compared to the controls. The lowest treatment matching 108 cm<sup>2</sup> lead per m<sup>2</sup> sediment surface (lead comprising 1.1% of the sediment surface) indicates that in stagnant waters this might lead to environmental concern.

### 2.14.P-We103 Drivers of Spent Gunshot Bioweathering in Wetlands

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Waterfowl hunting entails releasing large quantities of lead (Pb) gunshot into wetlands that can result in exposure and poisoning of wildlife, and environmental contamination. Pb shot remains in use for waterfowl hunting in many countries. Pellets deposited in wetlands are almost always submerged, with physicochemical and biological factors that result in bioweathering. We undertook a simplified *in vitro* approach to determine the extent to which organic matter and the presence of native microorganisms affected dissolution of major elements (Pb, Sb, and As) in shot. We examined the surface of gunshot by microscopy, and the efficiency and dynamics of weathering (proxied by pellet weight loss and elemental leaching efficiency) in different environmental conditions over a 60-day period. This study consisted of 4 treatment groups (10 replicates) that simulated some conditions that may be found in wetlands: 1) natural river water (RWA), 2) sterilized extract of pond sediments (SPS), 3) extract of pond sediments (APS), and 4) artificial root exudates (ARE). Elements were leached in proportion to their concentration in shot, with quantity of Pb being greatest, followed by Sb and As. Dissolution of Pb from shot differed among treatments. With the exception of the SPS and APS treatments, leaching of Sb and As differed between RWA and ARE treatments, but not between SPS and APS treatments. The greatest leaching was noted in the ARE group. One would presume that Pb, Sb, and As were homogeneously distributed in shot, and this was verified in part by findings of significant and strong relationships of total leaching among elements (Pearson correlation analysis,  $r > 0.9$  for all comparisons). We conclude that spent Pb shot has the potential to result in long-term contamination at some heavily hunted sites. The leaching of elements from spent gunshot can vary greatly depending on environmental conditions. Native microorganisms may play a stimulating role in weathering, but in comparison to other constituents of the matrix, this influence seems to be rather small. Such information will assist in the evaluation of the long-term risks posed by spent gunshot deposited on wetlands.

### 2.14.P-We104 Does Pre-Exposure to Sediment Spiked with Arsenic Influence the Effects of PMMA on *Hediste diversicolor*?

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In many coastal systems, sediment contamination is a relevant problem, where metal(loid)s can be found in higher concentrations than in the water column. These pollutants have been found in high concentrations in the tissues of marine invertebrates, altering enzymatic activities. For example, arsenic (As) is a toxic metalloid shown to impact behavioural and biochemical parameters, like neurotransmission and oxidative status, in polychaetes.

In the environment, organisms are exposed to a variety of contaminants, such as plastics, which have been a research focus in recent years. In marine ecosystems, they break down into micro- and nanoplastics (NPs); however, there are few studies on NPs effects in benthic invertebrates. Polymethyl methacrylate (PMMA) effects on marine organisms are still mainly unknown, particularly in polychaetes, which are key species with relevant roles in their environments. To the authors' knowledge, no studies have addressed the role of metal pre-exposure in worms' response to PMMA NPs. This study aims to understand how organisms living in contaminated areas may have their response to NPs modulated, assessing behavioural and biochemical endpoints in *Hediste diversicolor*.

Specimens of *H. diversicolor* were collected in a reference site of Ria de Aveiro and, after acclimation, 80 organisms were distributed in 8 aquaria and exposed, for 10 d, to 0 and 0.625 mg As/kg of sediment. Afterwards, 10 organisms per experimental condition were selected for the behavioural and biochemical assays, while 30 organisms from the control conditions and 30 from the As exposure were randomly distributed per experimental condition (0, 0.5, and 5 mg PMMA/kg) exposed for 10 d.

Behavioural data displayed no differences between conditions; however, data from PMMA NPs suggest a decrease in cholinesterase activity after the exposure to As. Superoxide dismutase was shown to be significantly increased in As pre-exposed organisms exposed to PMMA NPs, but not enough to prevent damage. Protein carbonylation was demonstrated to be significantly increased in pre-exposed organisms exposed to 0.5 mg PMMA NPs/Kg.

Obtained results demonstrated that As pre-exposure conditions influence the effects of PMMA NPs at the biochemical level. Thus, considering the increased concentration of plastics in marine environments, and the possible interactions of plastics with other pollutants, these types of studies should be performed considering the most common environmental contaminants.

#### **2.14.P-We105 Mercury in European River Sediments and Climate Change**

*John van Tol, Soil & Groundwater, TAUW bv, Capelle a/d IJssel, Netherlands*

Practically all EU countries have committed themselves to the minimization of mercury releases when signing the "Minamata Convention." Further obligations result from marine protection and EU regulations. According to the European Environmental Agency (EEA) mercury presents the biggest risk in rivers, lakes, and oceans where it is readily absorbed by animals and bioaccumulates in the aquatic foodchain.

The EU Water Framework Directive defines the chemical status of water bodies by complying with environmental quality standards (EQS) for selected chemicals. For many regulated parameters the EQS are exceeded only within a few water bodies. However, the situation for mercury is different. In Germany, for example, mercury presents a practically ubiquitous problem in surface waters. This is in line with findings from many EU member states: recent monitoring data show that out of approximately 111 000 surface water bodies in the EU nearly 46 000 are not meeting mercury levels set to protect fish-eating birds and mammals.

As for many persistent organic pollutants related to various activities in the catchment areas of Europe's large river basins, it is difficult to trace back the origin and the migration pathways of mercury. Historical primary sources are often abandoned. However, remaining soil and sediment deposits act as long-term secondary sources to the environment. For a number of (former) large industrial sites we have seen clear accumulation of mercury in river sediments. This is of great relevance as aqueous organisms are in direct contact with sorbed mercury sorbed in the sediment or dissolved in the porewater. Another threat is the further mobilization of particle bound mercury with flood events increasing both in frequency and in intensity due to climate change.

In our presentation we will discuss the fate and transport and the environmental risks of mercury in fluvial systems in Europe with particular regard to the increased environmental risks of heavy flood events. We will focus on systematic approaches for investigating historical sediment deposits in rivers, backwaters and floodplain soils and their dislocation in extreme flood events. We will illustrate this with a number of cases from sites in Germany, Spain, and the Netherlands. Detailed mapping and inventory studies in our opinion provide essential input for the following risk assessment and management steps in order to improve the chemical and ecological status of EU water bodies.

#### **2.14.P-We106 Assessing the Environmental Risk of Sediments from Areas of Natural Interest Through Applying Weight-of-Evidence Approach: A New Environmental Management Tool?**

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Aquatic sediments act as sinks for physical and biological debris, along with a variety of organic and inorganic pollutants. As they play a pivotal role in the functioning of aquatic ecosystems, assessing their ecological quality and pollution levels is essential for environmental management and marine biodiversity conservation. Yet, sediment quality monitoring, as implemented in most European directives, still relies on distinct assessments (e.g., physical-chemical, ecotoxicological, biological), lacking a unifying framework for a comprehensive analysis of the associated environmental risk.

Here, we applied an integrated approach to environmental risk assessment of sediment samples collected at Natura 2000 sites in the Gulf of Trieste (N Adriatic Sea, Italy) within the project MITFISH-N2K (FEAMP 1.40 - 2014-2020). The aim was to test

whether this approach (usually applied in potentially polluted contexts) may represent a new effective tool for monitoring the health status of sediments in protected areas.

The Weight-of-Evidence (WOE) approach proposed by Regoli et al. (2019) was used to build a multidisciplinary characterization combining different lines of evidence (LOE): the traditional lines of chemical characterization (LOE1) including 61 compounds (from "nonpriority" to "priority and hazardous" according to EC Directive 2008/105), the investigation of ecotoxicological properties of sediments (LOE4) through a series of bioassays following standardized protocols (involving algae, sea urchins, and amphipods), combined with the assessment of the ecological status of benthic communities (LOE5) based on the latest m-AMBI index.

LOE1 showed several compounds exceeding thresholds at nearshore sites (with corresponding moderate to very high-risk ratings) and low or no risks at more distant sites. No risk for any site emerged based on LOE4, suggesting the potential limitations of laboratory bioassays and underlining the need for an additional line of evidence to measure putative effects on resident populations (LOE5), which are the only biological components experiencing medium- and long-term exposure and adaptation processes. The outcomes of this study will provide useful insights on the potential of the WOE approach to become a new management tool for areas of natural interest.

#### **2.14.P-We107 Ecotoxicological Effects of Sediment Pollution in the Estuaries of Elbe and Odra**

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Estuaries are among the most productive ecosystems in the world and are critical to the life history and development of many aquatic species. Historic and ongoing sediment contamination remains a major concern for many estuarine sediments. Many pollutants (e.g., metals and POPs) adsorb to sediments and persist for long periods of time. Depending on their bioavailability, which itself may change with environmental conditions, these contaminant mixtures may exert adverse effects on aquatic organisms. Thus, it is crucial that sediment contamination in estuaries and its biological and ecological significance be properly assessed. One objective within the Blue Estuaries project (BlueEs) is to estimate the ecological stress from sediment-bound contaminants in the Elbe and Odra estuaries. For this purpose, surface sediments were sampled frequently at several sites over the course of two years. The sampling sites (8 in the Elbe estuary; 5 to 7 in the Odra Delta) were chosen along a presumed contamination gradient in both estuaries. To detect the ecotoxicological effects of the sediment samples, a biotest battery was applied comprising assays in direct sediment contact (*Arthrobacter globiformis*, *Caenorhabditis elegans*) and with elutriates (*Raphidocelis subcapitata*, *Alivibrio fischeri*, and *Daphnia magna*). The biotest responses were subsequently classified into different toxicity categories. In combination with the bioassays, an analysis of the meiobenthos composition (Nema-Spear-Index) was determined at the freshwater sites to assess the quality of the local biological communities. In addition to the determination of TOC and grain size fractions, the sediment samples were chemically analysed for heavy metals, 16 EPA PAHs, PCB, HCB, DDX, HCH which characterize the industrial history of both catchments. Preliminary results of the chemical analyses confirmed higher concentrations of historical contaminants in the Elbe estuary than in the Odra for all contaminants except PAHs which were higher in the Szczecin Lagoon. Highest ecotoxicological effects were observed with the algae growth inhibition test in the most upstream area of the Elbe Estuary and with the bacterial direct sediment contact test in the Szczecin Lagoon. An integrated assessment of chemical, ecological and ecotoxicological data will be carried out and presented in the poster.

#### **2.14.P-We108 Different Rivers, Common Problems – Linking Chemical and Ecological Status in Polluted Sediments of Three Different European River Basins**

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Contaminated sediments are still a major obstacle to achieve good chemical and ecological status in aquatic ecosystems, with the consequence that less than half of the EU's water bodies are in good status. Due to contaminant effects on sediment dwelling organisms, sediment-associated contamination contributes to disruption of the whole aquatic ecosystem because of benthic-pelagic food web coupling, which is a natural component of nutrient cycles influencing benthic and pelagic food webs and by that the ecological status. Weight-of-evidence (WoE) approaches, such as the sediment quality triade, using various lines of evidence (LoE) based on chemical, toxicological, and ecological information, can help to reliably determine sediment quality and to identify cause-effect relationships and thus allow a more effect-directed decision making in water management. However, there is a lack of specific pollution-sensitive metrics, and many macroinvertebrates in data of the EU water framework directive are not exclusively endobenthic and are to a large extent exposed rather to contamination in the water phase. These shortcomings may lead to misinterpretations of cause-effect relationships and, in the worst case, to inappropriate water management actions. Sediment-based LoEs (sediment quality guidelines; sediment toxicity tests; endobenthic bioindicators) might be more appropriate to reliably determine sediment quality and predict the risk of contaminated sediments for the aquatic ecosystem.

The EU Interreg project "Sullied Sediments" aimed to provide the tools for sediment assessment to enable better risk assessment and reduce economic costs. Therefore, contaminated sediments were sampled at three different river basins in Germany (Elbe), UK (Humber) and Belgium (Scheldt) over a period of 21 months (9 sites; 6 sampling campaigns) for analyzing physicochemical

properties, potentially toxic chemicals, ecotoxicity, and the endobenthic invertebrate fauna. As LoEs, the toxic potential based on sediment quality guidelines (LoE1), the ecotoxicity based on a toxicity test battery (bacteria, algae, nematodes) (LoE2), and chemical stress-sensitive biotic indices based on endobenthic macro-invertebrates (Biotic Sediment Index; BSI) and meio-invertebrates (NemaSPEAR[%]-index) (LoE3) were applied. The aim of this study was to evaluate the sediment quality triad as a tool for a more reliable and, thus, better decision making for water managers.

#### **2.14.P-We109 Integrated Assessment for Sediment Quality along the West Coast of Korea Based on Chemistry, Toxicology, and Ecology: Application of eDNA Analysis**

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Over the past few decades, the west coast of South Korea including various estuarine and coastal regions has been experienced environmental deterioration by persistent toxic substances (PTSs) due to industrialization and urbanization. We investigated the long-term changes of benthic microbial communities which adversely affected by PTSs (PAHs, styrene oligomers (SOs), nonylphenols (NPs), and heavy metals) and potential toxicities (AhR- and ER-mediated potencies and bioluminescence inhibition) in sediments from 10 sites of major estuarine and coastal regions in west coast from 2010 to 2018. Two perspectives were highlighted in this study, strengthening logistics of a comprehensive assessment in time (long-term; 2010–18) and space (inland vs. coastal comparison; for 5 regions). In general, compositions of bacterial communities varied spatiotemporally, but significant timely similarities were observed in community structure. Although bacterial compositions generally varied among or within regions, some phyla, such as Proteobacteria (29%), Actinobacteria (13%), and Chloroflexi (13%), were consistently predominant among locations. Alpha diversity of certain bacterial groups, such as Bacteroidetes and Firmicutes, revealed positive correlations ( $p < 0.05$ ) with concentrations of certain metals, including lead (Pb) and chromium (Cr). The diversity of bacterial communities was not significantly correlated with salinity, AhR-, ER-mediated activities, and concentrations of APs. Overall, the results suggested that the sediment bacterial community could be one potential proxy component to address the pollution status in the integrated sediment assessment.

#### **2.14.P-We110 Characterizing a Novel Passive Dosing Device Based on 3D Printing for Sediment Toxicity Tests**

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The ecotoxicological assessment of hydrophobic organic compounds (HOC) is connected to a series of difficulties. Due to their physicochemical properties HOC strongly sorb to organic material, test vessels, test organisms, and other surfaces. This might lead to an underestimation of the exposure concentration. Especially in sediment toxicity tests of HOC, the strong sorption might result in extremely low freely dissolved pore-water concentrations. For these substances, passive dosing (PD) is one suitable method, as it provides a reliable and constant exposure concentration of the test compound over the whole test duration. Potential losses due to sorption over time can be compensated through the inert reservoir loaded with the HOC and, thus, allows for full control of the pore-water concentration. PD reservoirs can be made from different materials (often silicone) and can have a variety of shapes. Typically, O-rings, tubes, or sheets are used. However, in sediment toxicity tests these standard shapes become problematic due to their small surface area and inhomogeneous distribution in the sediment. Therefore, in the present study, a complex form was designed *in silico* for a homogeneous distribution throughout the sediment, with a high surface-to-volume ratio ( $17.7 \text{ cm}^2/\text{cm}^3$ ) to ensure fast release kinetics. For the production of the device, a casting form was 3D-printed using a transparent, food-grade polylactic acid filament which was then casted with a food-grade, transparent 2-component silicone. The uptake kinetics of the model compound dodecylbenzene was determined as well as the release kinetics in pure water and in a water-sediment-system. The uptake of dodecylbenzene in the novel PD device was higher than in commonly used O-rings (20 % vs. 14.5 % (w/w)). This leads to the conclusion that the used silicone has a sufficient buffer capacity and might be a suitable material for PD. The loading time until saturation (6 hours) is comparable to that of O-rings. Next steps are the testing of different HOC and the adaptation of standard sediment toxicity tests with the new device.

#### **2.14.P-We111 Ecotoxicological and Chemical Assessment of Seasonally Collected Lake Suspended Particulate Matter**

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Monitoring suspended particulate matter (SPM) provides rich additional information on the sediment compartment in surface waters. In this project, we studied the quality of SPM with both ecotoxicological and chemical approaches over two sampling periods in a deep peri-alpine lake. We expected to see seasonal differences in link with lake dynamics, e.g. algal blooms and senescence or level of tributaries' particles input. To test this, we collected SPM in Lake Geneva over two successive periods of 4-5 months in sediment traps deployed at 30-m depth. After the integration period, trapping tubes were recovered and brought to the laboratory. Under standard conditions, we exposed laboratory-reared larvae of the insect *Chironomus riparius* directly in the tubes to assess effects on growth, emergence success and on the expression of over 15 biomarker genes. In addition, we conducted a standard mortality and growth inhibition bioassay with the ostracod *Heterocypris incongruens*. Lake SPM samples were characterized by physico-chemical analyses (grain-size, total organic carbon, S, N, and P contents and metal concentrations, including Hg) and submitted to nontargeted liquid chromatography–high resolution mass spectrometry (LC-HRMS). SPM-exposed larvae were digested for analysis of potentially bioaccumulated contaminants (e.g., metals, pesticides). While lake SPM of both sampling seasons inhibited chironomid emergence, larval growth was not impaired compared to the control. We observed

a strong effect on the ostracod growth for the first sampling and a severe mortality for the second. For the concentrations of metals in the exposed chironomid larvae, we did not see a difference between the two seasons. However, gene expression profiling was able to discriminate between samples from the first and the second campaign. By Orbitrap (LC-HRMS), we detected over 1000 compounds, 510 of which were common between the two seasons. Overall, thanks to both approaches, we could discriminate seasonal differences in the quality of Lake Geneva SPM. Quantitative analyses of targeted organic contaminants (ongoing) will provide a better understanding of the bioavailability of chemicals of concern in Lake Geneva. Further sampling campaigns will aim to decrease the period of integration of particles and enhance the frequency of the sampling to gain additional knowledge on the dynamics of lake SPM and the importance of the fate and effects of anthropogenic chemicals in aquatic ecosystems.

#### **2.14.P-We112 Sediment Avoidance Behaviour Test with *Lumbriculus Variegatus* as a Novel Screening Tool for Hazard Assessment**

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Sediments play an important role in the environmental partitioning of chemicals in aquatic ecosystems as they can act as a sink for lipophilic and persistent chemicals. This can lead to the exposure and bioaccumulation of substances in sediment organisms, key in aquatic trophic chains. Nevertheless, studies on sediment hazard assessment are still scarce, leading to the possible underestimation of the general chemical threat in the environment.

Hazard assessment is mainly based on short-term acute exposures using lethal endpoints as observed effects. However, more sensitive endpoints are necessary to improve our understanding of how chemicals impact our ecosystems. Behavioural assays represent an essential alternative, providing a faster and typically more sensitive response to chemical exposure than conventional sublethal endpoints like reproduction and growth. However, studies for behavioural assessment, especially for sediment-dwelling species and the oligochaete *Lumbriculus variegatus* in particular, are still scarce.

Avoidance behaviour tests are useful behavioural tools to assess the contamination of soils, linking individuals to the habitat function of soils. They represent rapid and cheap experiments to evaluate whether organisms perceive the presence of chemicals and escape to noncontaminated soils as a response. This study adapted the soil avoidance test design for the aquatic sediment compartment and tested it with *L. variegatus*. Three chemicals, a pharmaceutical, a pesticide, and a metal, known to be persistent in the sediment, were chosen for the experimental setup. Test boxes were divided into two compartments, where contaminated and uncontaminated sediments were placed on each side of the box. Ten *L. variegatus* were added where this division took place to give them an equal chance of choice. Data will then be used to calculate the avoidance percentage along with the capability of sediments to keep their habitat function. In this study, we show that these assays can be used to get a fast, accurate and easy-to-evaluate response to chemical stress using the sediment-dwelling species *L. variegatus*.

#### **2.14.P-We113 Biotransformation of Sediment-Associated Cyclic Volatile Methyl Siloxanes (cVMSs) by Benthic Invertebrates**

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Hydrophobic organic contaminants (HOCs), such as the cyclic volatile methyl siloxanes (cVMS, log  $K_{ow}$  >5), sorb to particles in the water column and eventually accumulate in sediments often to levels much higher than in the overlying water. The half-life of the most common cVMS in sediment is longer than in water: half-life; D4: 3.9 days in water, 365 days in sediment; D5: 70.4 days in water; 3100 days in sediment. However, results from our laboratory demonstrate lower half-lives in sediment inhabited by the estuarine polychaete, *Capitella teleta*, and the freshwater oligochaete, *Tubifex tubifex* due to a high biotransformation capacity. Deposit-feeding invertebrates, such as *C. teleta* and *T. tubifex*, colonize and thrive in organically polluted areas where they reach high densities (e.g., >100.000 pr m<sup>2</sup>). A high biotransformation capacity, as shown for several Persistent Organic Pollutants, may explain their ability to live in organically polluted areas. However, information on the mechanisms involved in the biotransformation processes is limited. Further, it has been found that *C. teleta* genome showed the presence of 96 functional cytochromes and xenobiotic response elements (XREs) following PAH exposure.

The aim of this study was to determine the mechanisms controlling biotransformation of sediment associated cVMSs by benthic invertebrates. The freshwater oligochaete, *T. tubifex*, and the estuarine polychaete, *C. teleta*, were exposed to sediment spiked with radiolabeled D4 (Octamethylcyclotetrasiloxane) for 28 days. We found no impact on survival, probably due to biotransformation of the parent compound to polar metabolites and a fast elimination. Furthermore, *C. teleta* and *T. tubifex* reduced sediment concentration of D4 faster compared to microbial degradation. ECOD and EROD *in vivo* analysis were used to assess the potential activation and increased activity of CYPs both in *C. teleta* and *T. tubifex* exposed to D4 to test the hypothesis that both species are able to break down sediment associated D4 through biotransformation.

#### **2.14.P-We114 Toxicity of Historic Oil Deposits on Marine Organisms: Preliminary Acute Toxicity Results**

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Oil pollution can be caused by any spillage of crude oil or its refined products. However, the largest and most damaging pollution events usually involve spills of petroleum or heavy 'bunker' fuel from collisions of tankers. The Greek tanker *Eleni V* was cut in two by the French vessel *Roseline* in foggy conditions off the southeast coast of England May 6, 1978. The collision caused the release of approximately 5000 tonnes of heavy fuel oil (HFO), polluting nearly 20 miles of the Norfolk coast and a significant amount of oil was buried in deep trenches in local beaches. In February 2021 Storm Darcy caused significant coastal erosion and re-exposed and mobilised these 40-year-old buried historic deposits. This study evaluates long-term environmental impact of residual oil from the event. Chemical analyses were performed to confirm residual polycyclic aromatic hydrocarbons (PAHs) still present in the sediment. Toxicity studies using the bacteria screening assay Microtox, the copepod *Tisbe battagliai* (48 hours mortality test), and the polychaete *Arenicola marina* (10 days sediment toxicity test) were performed to assess residual toxicity of the deposit.

Historic deposits were analysed for PAHs and alkanes by Gas Chromatography Mass Spectrometry (GC-MS). Results were compared with original *Eleni V* reference oil with samples showing 85% reduction of total PAHs and 70% of total alkanes. Acute toxicity results obtained with water accommodated fraction (WAF) for the bacterial assay Microtox and the copepod *T. battagliai* indicated limited toxicity of the deposits with EC50s > 100g/l. A 10 day *A. marina* sediment toxicity test was performed mixing oil deposits with reference sediment (up to 25%). Results showed no significant mortality compared to the control groups. Changes in feeding behaviour were observed indicating that the organisms were undergoing challenging conditions.

Acute toxicity results indicate that there is low likelihood of acute toxicological effects in both water column and sediment associated with the historic deposits. This is likely due to a reduction of total PAHs with time in the oil deposits compared to the original crude oil. Nevertheless, sublethal endpoints need to be further investigated to understand if organisms in the surrounding areas could be chronically impacted by the eroded deposits. More studies, on sediment dwelling organisms in particular, would be beneficial to exclude potential long-term impacts on organisms.

#### **2.14.P-We115 Organic Biomarkers as Indicators of Urban, Industrial, and Sewage Contamination in a Subtropical Estuary (Santos, SE Brazil)**

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Santos and São Vicente estuarine complex is one of the main estuaries of Brazil, with great economic and industrial importance, mostly related to the presence of the Santos Port and the Cubatão Industrial Complex, that include mainly chemical, metallurgical, and petrochemical factories. Over the years, the intense discharge of effluents from urban, industrial, and port activities have contributed to many environmental problems observed on the area. In this context, the assessment of the level of contamination of this environment was proposed, based on the evaluation of organic markers, such as aliphatic hydrocarbons (AHs), polycyclic aromatic hydrocarbons (PAHs), and sterols, in superficial sediment samples (n = 19) collected from different regions of the estuary and in the adjacent Bertioga channel. Results indicated higher concentrations of PAHs in the estuary (1176 – 32153 ng g<sup>-1</sup>), probably related to industrial activities, compared to the channel (534 – 6786 ng g<sup>-1</sup>). PAH distribution was similar among samples, with predominance of PAHs from pyrolytic sources that include oil and diesel combustion and vehicular emissions. AH results indicated petroleum contamination on all samples, with high concentrations of unresolved complex mixture (UCM) that oil is related to intemperized oil. N-alkanes distribution indicated mixed sources, with a strong signature of natural hydrocarbons (C25, C27, C29 and C31) from the adjacent mangrove vegetation, mainly in Bertioga Channel. Coprostanol indicated urban sewage contamination in all samples, with concentrations between 0.03 and 10.4 µg g<sup>-1</sup>. Although the environmental concern and efforts to control anthropogenic emissions into the marine environment have been increasing, the pollution by organic pollutants is still very relevant in one of the most important industrial and port areas of the Latin America.

#### **2.14.P-We116 First Assessment of (Anti)Estrogenic Activity in Sediment Core from a Mangrove in Rio de Janeiro State, Brazil**

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Paraíba do Sul River is very important to the water supply, industrial uses, and irrigation in Rio de Janeiro state (Brazil). It receives raw sewage, industrial wastewater, agricultural runoff, among others along its way. Mangroves are ecosystems of extensive relevance, and it is also a depositional environment. Sediments can act as sinks of contaminants and the geochronological study can provide valuable information on the contamination history and consequences of anthropic occupation. Bioassays have been used in sediment cores to assess paleotoxicology or to reconstruct each layer's estrogenic activity. However, assessing estrogenic activity in sediments is challenging because it is a complex matrix. Using an antiestrogenic assay allows



acknowledging if there are compounds in samples inhibiting the estrogenic activity by the YES (Yeast Estrogen Screen) assay. A sediment core was collected in a mangrove at the Paraíba do Sul estuary. It was sliced every 2 cm up to 20 cm and sectioned every 5 cm up to 1 m. This first analysis comprised the first 12 layers, up to 30 cm. Fine content (silt and clay), total organic carbon, and total nitrogen were determined. Samples were freeze-dried and extracted by sonication and solid-phase extraction. The estrogenic and antiestrogenic activities were assessed using the Yeast Estrogen Screen with *Saccharomyces cerevisiae*. To our knowledge, this study is the first screening assessment of estrogenic activity in a sediment core from a mangrove. The sediment core presented C/N molar ratio values of  $25.75 \pm 3.97$  and fine content of  $58.20\% \pm 4.91$ . Estrogenic activity was observed only in the first layer (0.09 ng/g Eq-E2). The antiestrogenic activity was qualitatively observed in eight layers, the most superficial ones, from 0 to 8 cm, and also in 10-12, 16-18, 20-25, and 25-30 cm. This suggests that antagonism might have masked the estrogenic activity of those layers. The chromatographic analysis will be held in the following steps to analyze which compounds are causing the antiestrogenic activity, as well as the dating analysis.

#### 2.14.P-We117 Chronic Exposure of Sediment-Associated Sertraline Leading to Endocrine Disruption in the Benthic Polychaetas *Capitella teleta*

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In the past few decades, the consumption of antidepressants has increased. When these pharmaceuticals are metabolized in the human body, a percentage of the parent compound and some metabolites are released to the environment via the down-the-drain pathway to the waste water treatment plants (WWTP). Thus, this is the main driver of antidepressants' contamination in the environment. Normally, antidepressants are detected in the range of ng L<sup>-1</sup> and µg L<sup>-1</sup>. Since they are designed to have a biological effect at low concentrations, they might pose a risk to native species, especially if exposure lasts for extended periods. Antidepressants, like sertraline (Log Kow 5.3), can sorb to particles in the water and subsequently accumulate in sediments with potential impact on sediment-dwelling organisms. Polychaetas are key organisms inhabiting benthic environments and, among them, *Capitella teleta* is widespread in estuarine organically enriched areas.

Since antidepressants are known to function as neurotoxic chemicals and endocrine disruptors, the aim of this work was to evaluate if sediment-associated sertraline has an impact on growth and reproduction in *Capitella teleta*. Polychaetas were exposed to five concentrations of sertraline (0, 0.3, 3.3, 33, and 100 µg/g dry wt), from day five posthatching until reaching maturation. Although not significant, worm growth rate showed a trend to increase when exposed to the lower concentrations of sertraline 0.3, 3.3, 33 µg/g dry wt. The increased growth was associated with a shorter time to reach maturity and time to first reproduction. Hence, our findings suggest that sediment-associated sertraline, at environmentally relevant concentrations, can disrupt *Capitella teleta* maturation and the timing of reproduction. Thus, studies on sediments-associated contaminants are fundamental to fully comprehend chemical's repercussions into the environments. Further research is needed to investigate the mechanisms underlying these effects.

#### 2.14.P-We118 Methods for Removal of Matrix Effect and Cytotoxicity in the Estrogen Activity Assay (YES) for Sediment Sample

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Pollution of aquatic ecosystems with emerging contaminants is a growing concern. Among these, endocrine disruptors (EDCs) are substances that can alter the endocrine system of living beings even at low concentrations. Its physicochemical characteristics indicate affinity with organic particles; therefore the study of detection methods in sediments is very important. Estrogenic EDCs can be evaluated by the *in vitro* Yeast Estrogen Screen (YES assay), which is based on the expression of the human estrogen receptor that has been incorporated into the yeast, *Saccharomyces cerevisiae*, genome. Despite being a well-established assay for chemical substances, this technique shows high interferences on the response and cytotoxicity of the yeast when associated with complex environmental matrices. For that reason, this study aimed to evaluate sediment sample preparation methods using solid phase extraction to remove cytotoxic compounds in the *in vitro* YES assay. It is being tested four methodologies for sample preparation. The first one (M-I) is based on the exclusive use of Strata-X cartridge on solid-phase extraction (SPE). The second method (M-II) is based on the use of EDTA for metal removal. Another methodology used (M-III) was based on clean-up sample using Strata-SAX cartridge on SPE. The last one tested (M-IV), is the association of both two last methods tested. All approaches used showed good viability on YES assay to quantify EQ-E2 (ng L<sup>-1</sup>). The M-I showed 0.68 ng L<sup>-1</sup> EQ-E2 while the cytotoxicity was 96%. On M-II, the Eq-E2 was 0.39 ng L<sup>-1</sup> expressing 95% of cytotoxicity, M-III 0.71 ng L<sup>-1</sup> Eq-E2 and no cytotoxicity was observed. The last method M-IV expressed 0.37 ng L<sup>-1</sup> Eq-E2 and no cytotoxicity. Concerning the numerical quantification of Eq-E2 on environmental samples, the last method tested showed a decreasing evaluation, which can be associated with some loss on the process. Even though M-III and M-IV were able to remove all cytotoxicity, the first one were considered better because it used less laboratorial insumes and showed more expressive response on EQ-E2. The isolated use of EDTA for metal removal was not enough to reduce all the cytotoxicity, while the SAX cartridge showed good results on cytotoxicity removal. It was concluded that the combined use of cartridges on SPE was the most viable technique for evaluating the estrogenic activity of sediment samples with the YES assay.

## 2.14.V New Developments in Sediment Ecotoxicology and Risk Assessment

### 2.14.V-01 RNA-seq Analysis of Transcriptome Response to Cadmium-Based Sediment Ecotoxicity using *Glyptotendipes tokunagai*

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High-throughput transcriptomics data have shown substantial information to disclose the complex molecular responses to pollutant toxicities. Here, chironomid's transcriptome analysis studied the ecotoxicological effects of cadmium (Cd), one of the prominent metal pollutants in the freshwater environment. The midges *Glyptotendipes tokunagai*, due to their relevance in the benthic sediments, opted for the insight on concentration and time-dependent molecular responses and tolerance mechanism to the toxic effects of Cd. We treated chironomids in sediments with three concentrations (low - 0.35 mg.kg<sup>-1</sup>, medium - 3.35 mg.kg<sup>-1</sup>, high - 10.70 mg.kg<sup>-1</sup>) of cadmium chloride (CdCl<sub>2</sub>) for 1-10 day intervals. We observed evident changes in the gene expression and pathway regulations comparatively higher at the high Cd concentration and longer duration (10 days). The complete transcripts represented 10275 significantly expressed genes with an average length of 795 bp. Gene set enrichment analysis indicated the progression of differentially expressed genes (DEGs). Cd exposure in 1-day duration pronounced upregulated genes in autophagy and signal transduction which mediates ion homeostasis, receptor binding, and cytoskeletal protein regulation. Further, downregulation in metabolic pathways intervenes in molecular functions. Extended exposure (10 days) tends to the upregulated metabolic pathways, cofactors biosynthesis, cuticle metabolism, and detoxification response inducing glutathione metabolism and catalytic activity. Network mapping using the STITCH database predicted the interaction of encoded proteins of stress response and tolerance mechanism reliable to the effect of Cd toxicity in the sediments. The transcriptome of chironomids portrayed the signal transduction-based cellular regulation, and stress response in 1 day samples, further adapting the tolerance mechanism via detoxification phases to withstand prolonged toxic effects.

### 2.15.P Pollutant-Induced Perturbations of Host-Associated Microbiomes as Both Indicators and Modulators of Environmental Toxicity

#### 2.15.P-Th117 Fungicide and Insecticides can Alter the Microbial Community on the Cuticle of Honeybees

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Honeybees are crucial for our ecosystems as pollinators, but the intensive use of plant protection products (PPPs) in agriculture poses a risk for them. PPPs do not only affect target organisms but also affect nontargets, such as the honeybee *Apis mellifera* and their microbiome. This study is the first that aimed to characterize the effect of PPPs on the microbiome on the cuticle of honeybees.

We chose PPPs which have frequently been detected in bee bread and studied their effects on the cuticular microbial community and function of the bees. The effect of the fungicide Difcor®, the insecticide Steward®, the combination of both (mix A) and the fungicide Cantus® Gold, the insecticide Mospilan® and the combination of both (mix B) were tested. The honeybees (*A. mellifera carnica*) were collected from a hive of the departmental apiary at the University of Würzburg and transferred into cages. Feeding solutions were provided via prepared 2 mL cups. Doses were chosen based on their corresponding residue values found in the field. Feeding solution consists of 30% sugar water and addition of respective PPP. After nucleic acid extraction from the bees' cuticle, bacterial 16S rRNA gene and fungal transcribed spacer region gene-based amplicon sequencing were carried out. Additionally, quantification of bacterial and fungal gene copy numbers were done by quantitative PCR using the same primers. The treatment with Steward® significantly affected fungal community composition and function. Bacterial cuticular community composition of bees treated with Cantus® Gold, Mospilan® and PPP mix B differed significantly from the control. In addition, Mospilan® on its own significantly changed the bacterial functional community composition. Cantus® Gold significantly affected fungal gene copy numbers, community and functional composition. Our results demonstrate that PPPs show adverse effects on the cuticular microbiome of *A. mellifera* and suggest that PPP mixtures can cause stronger effects on the cuticular community than a PPP alone. This may have far reaching consequences for the health of honeybees.

#### 2.15.P-Th118 Copepod-Associated Microbiome as Biosensors of Pollutant Exposures in the Marine Environment

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Trace amounts of thousands of manmade organic pollutants (OPs) and hydrocarbons reach seawaters mainly through run-off, riverine, and diffuse atmospheric inputs. The fraction of marine dissolved organic matter (DOM) accounted by OPs remains unknown due to the analytical complexity to analyse both pools. However, this assessment is important because OPs are hydrophobic, and thus tend to accumulate in marine biota at higher intracellular concentrations than in seawater. Copepod-associated bacterial communities can be used as natural sensors of OPs exposures although is a subject seldom studied. Previous works have described copepod-associated microbiomes in different marine environments and locations, and although there is

some research on the factors regulating copepod-associated microbiome, they are mostly focused on their role on microbial production, biogeochemical cycles and diversity maintenance. The aim of this research is to study how OPs and other different environmental factors modulate copepod-associated microbiomes (size of copepod, location, physico-chemical conditions) in an Atlantic Ocean transect, in order to detect specific taxa in the microbiomes that can be used as natural biosensors of pollutant exposures in the marine environment. Samples of copepods and seawater were collected during an Atlantic Ocean transect crossing latitudes between Vigo (Spain) to Punta Arenas (Chile). DNA was extracted and 16S rDNA was sequenced using a paired-end 250 Illumina system to characterize copepod-associated and seawater microbiomes, and pollutants were measured in the seawater from the same sampling locations. Taxonomic composition of copepod-associated microbiomes was dominated by *Gammaproteobacteria*, *Actinobacteria*, and *Alphaproteobacteria*; remarkably, *Vibrio* spp. were present at high abundances, mostly in big copepods and northern/central locations. Significant differences were seen in the bacterial communities of big and medium size copepods, and between different stations. Copepod-associated microbiome was different to that of the seawater, and shared more taxa with particle-attached microbiomes compared to free-living bacteria. During the next months, changes in community structures in copepod-associated microbiomes will be correlated to *in situ* concentrations of selected organic pollutants and other environmental parameters relevant in the marine environment to detect potential taxa of interest of marine OPs exposures.

#### **2.15.P-Th119 Microbiota Alterations in Three Passerine Birds Under Environmental Metal Exposure**

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Industrial activities such as smelters emit various heavy metals into the environment. These metals are known to affect the physiology and fitness of wild birds. However, the indirect effects of metal exposure on bird health via changes in the microbiota have not been studied. As microbiota plays a vital role in bird health, there is a need to investigate whether microbial composition can be altered by metal pollution. To address this knowledge gap, we observed the effects of anthropogenic metal pollution on the nest and gut microbiota of three common passerine birds: great tits (*Parus major*), blue tits (*Cyanistes caeruleus*), and pied flycatchers (*Ficedula hypoleuca*). These species were studied near a copper-nickel smelter, where high levels of Cu, Ni, As, Cd, and Pb are found in the environment. We inspected 30 nests per study species from metal-polluted and unpolluted areas to collect data on breeding variables. Furthermore, we took nest swabs and nestling fecal samples, the latter of which were split for microbial (16S rRNA sequencing) and metal (ICP-OES) analyses. Our preliminary findings suggest that there were no significant differences in the microbial alpha diversity of the nests between species or pollution groups. However, it seems that the microbial community compositions ( $\beta$ -diversity) in the nests differed between polluted and control sites, depending on bird species. Differential abundance analysis indicates that the abundance of Proteobacteria may be decreased in the polluted nests of blue and great tits, but not in pied flycatchers. The observed differences in the nest microbial communities between bird species and pollution groups may be explained by differences in metal tolerance between bacteria and variations in nest materials. In the future, it is necessary to perform experimental manipulations with metal mixtures to entangle the possible causality between metal pollution and microbial composition. Further investigations are required to determine the consequences of metal exposure for wild avian health and fitness via microbiota alterations.

#### **2.15.P-Th120 Effects of Agricultural Management on the Gut Microbiota of the Iberian Hare**

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The gradual shift from traditional agriculture toward more mechanized market agriculture that took place during the 20th century has led to a major change in the traditional agricultural landscape worldwide. This agricultural intensification, focused on increasing crop yields, has led, on the one hand, to an important homogenization of the landscape due to land consolidation and, on the other hand, the entry of more and more pesticides and fertilizers in the environment. In broad terms, this agricultural intensification has been associated with the loss of plant diversity, as well as a significant decline in wildlife species associated with agrosystems worldwide. In the case of pesticides, although their mode of action is often known in the target species, their secondary or sub-lethal effects on wildlife are still unknown. We face, therefore, the need to causally link knowledge about the molecular actions of pesticides to their possible interference with biological processes, in order to develop reliable predictions about the consequences of the use of pesticides. Among the effects of pesticides, a growing number of studies in recent years have described their potential to alter the composition of the host-associated gastrointestinal microbial communities, which in turn contributes critically to a variety of host metabolic and immune functions. Effects that may have an impact on animal health, and thus represent key modulating factors at the population level. Using the Iberian hare (*Lepus granatensis*) as study model, this work was aimed to study the effect of agricultural management based on the use of pesticides on the gut microbiota. For this purpose, the microbial community of hares hunted in pesticides-treated and pesticides-free areas during the 2018/19 hunting season in the central Spain was studied in gut samples. Our results showed that agricultural management is capable of altering the gut microbiota, suggesting that more research is needed to understand the potential repercussions of this alteration at the population level.

#### **2.15.P-Th121 Effects of Zinc Contamination on Arbuscular Mycorrhizal Fungal Function in Urban Horticulture**

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By 2050 the global population is likely to be approaching 10 billion people, 70% of whom are expected to live in cities. Food

production needs to increase in order to meet demand; however due to climate change, agricultural land currently in use may become unsuitable for crop production. The utilisation of urban soils to increase food production at a local scale may have a role to play in contributing to food security in the future. However, the soils in urban allotments, which have become increasingly popular in recent times, are susceptible to high levels of Zn deposition from industry and transport, as well by land management practices such as the application of manures and fertilisers. Zn is an essential micronutrient for plant growth and development but at high concentrations, Zn can have detrimental impacts on plant health and productivity. Additionally, the prevalence of Zn in the soil is thought to affect soil health by influencing the diversity of the soil microbial communities, including symbiotic microorganisms such as arbuscular mycorrhizal fungi (AMF). AMF form symbioses with the roots of most plants and can improve host plant resistance to stresses such as heavy metal toxicity while simultaneously increasing access to soil nutrients such as inorganic phosphorus and water, in return for host plant-fixed carbon. However, the impact of the distinct properties of allotment urban soils on the functionality of AMF has remained uninvestigated. By measuring key plant traits and tracking AMF function using isotope tracers, we investigated the effects of environmentally relevant Zn concentrations in soil on the phenotypic characteristics of the plant and resource exchange between peas and AMF. Our results show Zn may affect the function of AMF in urban soils, and the subsequent yields of crops grown in urban environments, as well as how the symbiosis between AMF and the plant may allow the plant to cope better with the additional heavy metal stress.

#### **2.15.P-Th122 The Gut Microbiome Causally Contributes to Interspecific Differences in Pesticide Sensitivity**

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Explaining interspecific differences in pollutant sensitivity is key to increase the predictive power of ecotoxicology. Recently, not only organismal traits, but also the gut microbiome has been hypothesized to be a potential factor in shaping host tolerance to pollutants. This generates the hypothesis that the gut microbiome may provide an additional predictive factor of pollutant sensitivity, complementary to the trait-based approaches in ecotoxicology. Therefore, we investigated the causal role of the gut microbiome in shaping differences in pesticide tolerance between two conspecific damselfly species. A reciprocal gut microbiome transplant experiment was performed between larvae of the less pesticide-tolerant *Ischnura elegans* and the more tolerant *I. pumilio*. Subsequently, recipient larvae were exposed to the organophosphate insecticide chlorpyrifos or a solvent control. The gut microbial community of both species included pesticide-degrading bacteria, and showed besides fixed species differences also species-specific responses to the pesticide. Consistent with our hypothesis, the gut microbiome contributed causally and predictably to the difference in tolerance between both species. Indeed, the most sensitive donor-recipient combination to the pesticide was when *I. elegans* recipient larvae received an *I. elegans* donor gut microbiome, whereas the least sensitive combination was when *I. pumilio* recipients received an *I. pumilio* donor gut microbiome. The mixed donor-recipient microbiome combinations had an intermediate tolerance. Our results therefore provide the first proof-of-principle that the gut microbiome may serve as an additional predictive factor of species differences in pollutant sensitivity next to species traits in ecotoxicology.

#### **2.15.P-Th123 The Role of the Host-Associated Microbiome in Host Tolerance and Cross-Tolerance to Pollutants**

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Populations that are exposed to a pollutant can evolve tolerance to this pollutant. Tolerance to one pollutant can increase tolerance to another pollutant, so-called cross-tolerance. Understanding patterns of cross-tolerance is important as populations are increasingly exposed to multiple pollutants. While host mechanisms of (cross-)tolerance are well studied, it remains untested whether the host's microbiome may also play a role in driving cross-tolerance by lowering the toxicity of pollutants for the host. In this study, we therefore studied the host-associated microbiome community as underlying mechanism of its host's (cross-)tolerance. We build on an experimental evolution trial in which *Daphnia magna* evolved tolerance to the pesticide chlorpyrifos which was associated with cross-tolerance to the pesticides malathion and carbaryl, while it did not change the tolerance to the pesticide esfenvalerate and reduced the tolerance to the pesticide imidacloprid and the pharmaceutical fluoxetine. We determined the whole body microbiome community composition of two chlorpyrifos-sensitive clones and two chlorpyrifos-tolerant clones after 4-days exposure to a control treatment or a sublethal concentration of one of the above pollutants. In the control treatment, the chlorpyrifos-sensitive and -tolerant clones had a different bacterial community composition supporting the idea that changes in the microbiome contributed to the evolution of tolerance to chlorpyrifos in the host. While exposure to the organophosphates chlorpyrifos and malathion did not change the bacterial community composition of the chlorpyrifos-tolerant clones, it did cause a compositional change in the bacterial community of the chlorpyrifos-sensitive clones, suggesting that the chlorpyrifos-tolerant clones have a microbiome with bacteria that can thrive under organophosphate exposure as they can degrade these pesticides. These bacteria may contribute to the host's tolerance to chlorpyrifos and cross-tolerance to malathion. Our study hereby provides the first experimental indication that the host-associated microbiome plays a role in not only the host's tolerance but also cross-tolerance to pollutants. In addition, our results show pollutant-specific shifts in the microbiome community composition whereby the microbiomes of both chlorpyrifos tolerance types were strongly affected by exposure to carbaryl and imidacloprid, yet not significantly affected by exposure to esfenvalerate and fluoxetine.

#### **2.16 Soil Function and Biodiversity: Impacts and Resilience Under Stressed Environments**

##### **2.16.T-01 C and N Mineralization in Metal Mine Tailings Technically Recovered versus Spontaneously Colonized by Vegetation as Indicator to Assess Soil Functionality**

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Metal mine wastes from ore processing are among the most hazardous residues (e.g., high metal(loid) levels, extreme pH, high salinity, organic matter/nutrients deficiency). These wastes are often deposited in open-air piles (mine tailings). Classic-restoration approaches (technical reclamation) usually involve topsoil capping aiming to create a “new soil” above wastes and then afforestation. These options have been questioned in (semi)arid regions due to ecosystems inability to be self-sustaining. An alternative, based on nature-based solutions principles, is to promote the growth of plants directly in mine wastes (phytomanagement by phytostabilization). This contributes to create the so-called fertility islands that may promote plant-soil feedbacks and ecosystem functioning. If tailings pose immediate risks (e.g., structural collapse, close to urban areas), the necessary financial/technical means should be made available for immediate technical restoration. However, if conditions are not so extreme, the spontaneous succession of plants directly growing in mine wastes has some advantages (e.g., plants adaptation to local climate/substrate conditions, high natural value, improved long-term sustainability). Previous works showed that the direct growth of native plants in tailings can provide functional soil-vegetation systems. This study aimed to evaluate whether technically reclaimed (capping and afforestation) and spontaneously colonized metal(loid) mine tailings’ soils differ on carbon and nitrogen transformation activities. For this, C and N mineralization rates (following OECD guidelines) were evaluated in soil samples collected in spring 2022 in areas with both shrubs and pine trees in two mine tailings from SE Spain: a tailing technically reclaimed by capping and afforestation, and a tailing spontaneously colonized by plants directly growing in mine wastes. The results showed that both tailings differed in their C transformation activity, showing the technically reclaimed tailing greater capacity to mineralize C. However, in the case of N, both tailings showed similar mineralization rates. Furthermore, the soils under shrubs were the ones reaching greater mineralization scores in the technically reclaimed tailing, while the contrary occurred in the tailing spontaneously colonized by vegetation with greater mineralization scores in soils under pine trees. The latter may suggest that the type of vegetation is modulating soil functioning.

#### **2.16.T-02 Biocides Containing Façade Eluates Alter Soil Microbial Community Composition and Activity**

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Combinations of biocides are commonly added to building materials to prevent microbial growth and thereby cause degradation of the façades. These biocides reach the environment by leaching from façades and pose an environmental risk. Studies regarding the effects of those on the soil microbiome are relatively scarce. Our previous study showed that combined entry of biocides frequently used in building materials into the soil had tremendous effects on active soil microbial communities and their associated functions, even though overall soil parameters such as soil respiration and soil physicochemical parameters were unaffected.

This study aimed to characterize the effect of façade eluates on the soil microbial community composition and function. Therefore, eluates from samples without biocides and samples containing both in-can and film preservatives were generated within a natural weathering experiment and introduced to soil microcosms outdoors. After the leaching experiments, the eluates were diluted since only a few percent of the horizontal rainfall is biocide-containing façade runoff. To resemble the realistic scenario at the buildings, eluates were added with every rain event to the soil microcosm. Samples were treated with Bromodeoxyuridine (BrdU) one day before sampling to mark active proliferating members of the soil microbiome. After 29 and 53 days of incubation samples were drawn. Those samples were investigated by BrdU immunocapture technique in combination with pair-end Illumina sequencing to differentiate the total and active bacterial and fungal microbiomes. Bacterial and fungal gene copy numbers were quantified via Real-Time PCR analysis. Moreover, soil chemical parameters, soil respiration, biocide, and biocidal degradation product concentrations were determined and compared to the control (incubation with rain water). The resulting data set is then analyzed using multivariate statistics. In addition, a functional annotation for bacteria and fungi is conducted using the FungalTraits and FAPROTAX database.

No effects on soil physicochemical parameters and overall soil microbial activity could be observed. BrdU immunocapture technique in combination with pair-end Illumina sequencing is currently in progress and will show if there are any species-specific effects on active and total soil microbial community. We suspect that, similar to the last study, changes in the microbial community were also caused by the treatment with eluates.

#### **2.16.T-03 Increased Temperature Enhances Toxicity From Contaminant Stress in a Widespread Collembola Species With Different Thermal Adaptation**

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Springtails (Collembola) are important components of soil ecosystems worldwide, and may be exposed long-term to anthropogenic contaminants. As global air temperature continues to increase, it is likely that it will affect ectotherms’ responses to contaminant stress. Populations inhabiting different climates may respond differently to higher temperature, as their adaptation to utilize ambient heat likely differ. Our aim was to document whether sublethal effects on Collembola exposed to an insecticide depend on the ambient temperature. Additionally, we aimed to determine effect from the thermal adaptation of the study organisms on their response.

One temperate and one arctic originated population of *Hypogastrura viatica* (Collembola) was exposed dietary to two sublethal concentrations of imidacloprid and a control, at 10, 15, 20, or 25°C. The exposure lasted from 0 to 1500 day-degrees (days\*temperature), allowing comparisons between temperatures. Each experimental unit contained 25 juveniles, and each treatment had 12 replicates, of which 4 were harvested at three different life stages. We studied among other the traits survival, growth (body size) and egg production.

Dietary exposure to imidacloprid resulted in sublethal effects for both populations at 10 and 15°C (>85% survival), while the highest concentration of 0.16 mg/kg dry bark was lethal at 20°C and 25°C (approx. 50-70% survival). The effect was stronger for the temperate population. The growth pattern for both populations showed little or no effect on the body size at an early age, but a strong reduction in body size at a high adult age, which was increasing with increasing temperature. A decrease in egg production in arctic *H. viatica* was caused by imidacloprid exposure of the highest concentration, but without any apparent interaction from ambient temperature. Contrastingly, the toxic effect on egg production was temperature-dependent for temperate *H. viatica*, for which higher temperature resulted in a stronger decrease in number of eggs laid.

Unlike the arctic population, the temperate population is adapted to efficiently utilizing a warm summer climate by increasing egg production at increasing temperature. The energy cost of high egg production might cause the temperate population to be more sensitive to other stressors when the temperature is high. Knowledge on how other factors affect toxicity from contaminant stress can contribute to a more relevant risk assessment in the future.

#### **2.16.T-04 Eco-Indicators Sensitivity Distribution (EcoSD): A Systematic Approach to Redefining the Species Sensitivity Distribution (SSD) for Ecosystem Services Approach in Soil Ecological Risk Assessment**

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Soil ecological risk assessment is usually based on toxicological endpoints of individual soil invertebrates and plant species, with little consideration for soil ecological processes. For example, the Species Sensitivity Distribution (SSD) is helpful in the tiered ecological risk assessment (ERA) approach. Still, this approach needs to be improved by considering key soil ecological processes that are directly linked to ecosystem services. The ecosystem services (ES) approach has gained recognition because it directly relates the effect of environmental stressors on service providers to human wellbeing. Therefore, this study aims to propose and describe a concept, the Eco-indicator Sensitivity Distribution (EcoSD), that employs data from ecological indicator responses to chemicals in deriving ecotoxicity thresholds that will protect vulnerable ecosystem processes and services in a terrestrial ecosystem. The EcoSD is like an SSD but based on ecological indicators rather than individual species. EcoSD is conceived to fit into the existing ERA as an initial step of the tiered approach, accommodate site-specific assessment for different land use, and incorporate a dose-response approach to deriving an ecologically safe limit or threshold to protect the ecosystem.

#### **2.16.T-05 Upcycling Agricultural Co-Products to Improve Soil Quality and Ecosystem Services in Sustainable Agriculture**

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During the last decades, a Green Revolution took place in agriculture, triggered by significant advances in scientific knowledge and characterized by fast increases in production yields, essential for a flourishing population. Still, this intensification in agricultural production has led to environmental deterioration, imperiling the delivery of ecosystem services. Moreover, food supply chains also face an increasing problem of food loss and waste, further contributing to environmental unsustainability. Therefore, a new revolution is urgent to ensure food security, one that is rooted in novel sustainability paradigms such as efficiency and circularity. Technological innovations focus on the use, transformation, and valorisation of agroindustrial byproducts into innovative products aimed to ameliorate soil functioning, reduce fertilizer dependence, and maintain ecosystem integrity, such as agro-waste-based fertilizers (AWBF). Bearing this in mind, our study used an ecosystem services approach to evaluate the effectiveness and environmental impact of an AWBF derived from the digestion of vegetable co-products by *Hermetia illucens* larvae (product under development). In a greenhouse production system, a randomized block experiment with four treatments was established: 1) reference (no fertilization), 2) NPK plus horse manure, 3) NPK plus AWBF, and 4) AWBF alone. An integrated assessment included: tomato productivity and quality (provisioning services), mesofauna and microbial communities biodiversity (regulating services), functionality of microbial communities (supporting services), *in situ* feeding activity of soil organisms (supporting services), and soil retention function (regulating services). Overall, our results showed that tomato yield was significantly higher in the soil amendments with NPK and manure and AWBF alone. The feeding activity of soil fauna did not differ between treatments and low average percentages of feeding activity were observed, which can be related with lower abundance of soil fauna. Nevertheless, a stimulatory effect in soil enzymatic activity was observed, in the case of AWBF and NPK and AWBF-treated soil. As for soil retention function, AWBF did not pose risk to freshwater organisms. Our multidisciplinary approach showed that AWBF might be considered an appropriate and environmentally safe fertilization management option for tomato production, improving the sustainability of terrestrial and aquatic ecosystems.

## 2.16.P Soil Function and Biodiversity: Impacts and Resilience Under Stressed Environments

### 2.16.P-Mo142 Arbuscular Mycorrhizal Fungi as Microbial Indicators to Characterize Soils and Their Use Intensity

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Arbuscular mycorrhizal fungi (AMF; Mucoromyceta) are an important component of fertile soils, as they support several key soil functions: for instance they improve plant growth and health, stabilize soil aggregation, enhance rainwater infiltration, and retain macro- and micro nutrients in the upper soil layers. Currently, approximately 350 AMF species are known worldwide. This number is steadily increasing, since new molecular technologies allow identifying new as yet undescribed species. During the last 20 years, we studied the diversity of these fungi in a series of natural or agricultural soils, in different continents, under different climatic conditions, at different altitudes, and subjected to different conventional or organic farming and soil tillage practices. We found that worldwide multiple AMF species as well as the overall AMF community structure can be used as powerful indicators in various aspects such as agricultural sustainability, soil fertility, water and nutrient budgets, or soil degradation due to overuses or climate change. About 2-80 species were detected in natural and agricultural soils depending - among other factors - on climate, land use type and intensity, plant species diversity and specific soil parameters. In arid soils, only Glomeraceae, Diversisporaceae, or Paraglomeraceae species were detected. Under semiarid to humid conditions, also Acaulosporaceae and Gigasporaceae species occur, but they are generally more sensitive to high fertilization levels than Glomeraceae species, and often they are less common in neutral to alkaline soils. In a wide range of soils types, e.g. ,Tschernosems, Luvisols and Ferralsols, a high AMF diversity can be found even under intensive agricultural production, as long as the majority of the fungi have suitable living conditions during the vegetation periods. Herbicide and even repeated fungicide applications might then have only minor effects on the AMF communities in the soils. Their diversity can, however, be dramatically decreased, especially when the plant root and hyphal network is periodically disturbed by harsh tillage practices, when plants suffer due to increasing droughts, or when specific weeds or entire weed communities are periodically eliminated by chemical measurements.

### 2.16.P-Mo143 Evaluation of Antibiotics and Copper Mixture Effects on Test Organisms, Natural Soil Microbial Communities, and Plant Growth: An Ecological Study at Different Hierarchical Levels

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Livestock manure is a common used organic fertilizer; however, recent investigations have demonstrated it can be a source of heavy metals (e.g., copper) and antibiotics (ABs) for agroecosystems. Copper (Cu) is used in organic farming at high concentrations (30-800 mg/kg) as antifungal, and toxic effects to biota are not excluded. For this reason, agricultural soils can be environmental sinks of ABs and Cu, which can be accumulated in plants and eventually pass through the food chain to animals and humans. AB presence in soil can exert a selective pressure which promotes antibiotic resistant bacteria spread and related resistance genes.

The aim of this work was to increase knowledge on this issue, using an ecological approach at various hierarchical levels. Firstly, ecotoxicological standard tests were performed, with *Aliivibrio fischeri*, *Lepidium sativum*, and *Daphnia magna*, to evaluate acute effects of copper and antibiotics (alone or in mixture) in terms of effective doses (e.g., EC<sub>50</sub>). Subsequently, soils amended with manure/digestate and spiked with copper alone (30 mg/Kg) or in combination with three common antibiotics (sulfamethoxazole and ciprofloxacin, 7.0 mg/kg each) in presence/absence of *Lactuca sativa* were studied in microcosm experiments. The effects on natural soil microbial communities were evaluated in terms of abundance (DAPI count), viability (% Live cells /Live+Dead), enzymatic activity (Dehydrogenase activity) and community structure (NGS). Moreover, plant growth (biomass of roots and aerial part) was evaluated.

AB effective concentrations (SMX, EC<sub>50</sub>:194.06 mg/L and CIP EC<sub>50</sub>:250.10 mg/L) were higher than environmental ones. The EC<sub>50</sub> found for Cu (0.78 mg/L) was significantly lower than concentrations found in agroecosystems, suggesting a risk for natural biota. Moreover, additive detrimental effects were recorded in ABs and Cu mixtures.

Soil natural microbial communities were partially affected by Cu presence; however, in organic amendments and plant co-presence, microbial communities were not affected by copper toxicity. Microbial community structure analyses evidenced significant differences between different conditions. Analyses of ABs and Cu bioaccumulation in lettuce tissue are in progress.

### 2.16.P-Mo144 LDPE and Biodegradable Plastics Differentially Affect Plant-Soil Nitrogen Partitioning and Dynamics in a *Hordeum vulgare* Mesocosm

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Micro- and macroplastics are emerging contaminants in agricultural settings, yet their impact on nitrogen (N) cycling and partitioning in plant-soil-microbial systems is poorly understood. Furthermore, the long-term impact of increasing macroplastic and microplastic loading from plastic mulching on ecosystem services is relatively unknown. This includes the microbially mediated N transformations, which is central to agricultural productivity. In this mesocosm-scale study, spring barley (*Hordeum vulgare* L.) was exposed to macroplastic (1 cm) or microplastic (<500 µm) produced from low density polyethylene (LDPE) or biodegradable (PLA/PBAT 15%/85% w/w) plastic mulch at concentrations equivalent to 1, 10, and 20 years of plastic mulch film use. Partitioning of <sup>15</sup>N-labelled fertiliser into plant biomass, soil, and leachate yielded a partial mass balance. Soil-N partitioning and microbial assimilation was probed via compound-specific <sup>15</sup>N-stable isotope analyses of soil microbial protein. Dose-dependent decreases in plant <sup>15</sup>N uptake occurred with increased leached nitrogen for LDPE microplastic, due to changes in physical pore flow pathways. Assimilation into soil microbial protein was higher for biodegradable plastics, which we associate with early-stage biodegradable plastic degradation. Microbial assimilation in the presence of LDPE was a function of abiotic impacts on leaching, with suppression of inorganic N transformations. While microplastics and macroplastics altered soil N cycling, the limited impacts on plant health indicated the threshold for negative effects was not reached at agriculturally relevant concentrations. However, changes in soil N cycling and available N will impact nitrogen use efficiency and soil organic matter dynamics. Thus, the differing impacts of conventional and biodegradable macro and microplastics must be considered in risk assessments for agricultural plastics.

## **2.16.P-Mo145 Adverse Effects of Agrochemicals on Methanotrophy Examined in a Toxicity Assay with Methane Oxidizing Bacteria**

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Biological methane oxidation by high-affinity methanotrophic bacteria in soils is the only known biological process that can remove methane (CH<sub>4</sub>) from the atmosphere. Methanotrophic bacteria play a vital role in the global carbon-cycle and could potentially mitigate increases in global methane emissions. Aerobic methanotrophic bacteria belong to a small number of bacterial lineages, and a key characteristic is the synthesis of methane monooxygenase enzymes which catalyze the oxidative attack of the otherwise stable CH<sub>4</sub> molecule. Methane monooxygenases are unique and enable methanotrophic bacteria to grow on CH<sub>4</sub> as their sole source of carbon and energy. Due to their unique cellular metabolism, methane oxidizing bacteria are sensitive to a number of environmental disturbances including natural and anthropogenic toxicants. This study developed a new toxicity assay to investigate adverse effects of toxicants on aerobic methanotrophic bacteria, and screened common agrochemicals for effects on methanotrophy. A range of methanotrophic bacteria were screened for responsiveness and the facultative methanotrophs *Methylocapsa aurea* and *Methylocystis hirsuta* were selected for a toxicity assay with methanotrophic bacteria (TOX-MOB). TOX-MOB is based on growth and activity measurements in 96-well microplates after incubation with CH<sub>4</sub> and the endpoints are growth rates, electron transfer system (ETS) activity and production of reactive oxygen species (ROS). Ten concentrations of each compound were examined and 18 potential toxicants were examined including antibiotics, pesticides, biocides, and metals. The responses of *M. aurea* and *M. hirsuta* were compared with results obtained in parallel experiments with the bioluminescent reference bacterium *Aliivibrio fischeri*. The results showed that EC<sub>10</sub>, EC<sub>20</sub>, and EC<sub>50</sub> values obtained in the TOX-MOB assay were lower for 16 of 18 test compounds suggesting that methanotrophic bacteria are inhibited at lower concentrations than standard bacterial test organisms. For example, growth and energy metabolism in methanotrophic bacteria were affected by elevated concentrations of compounds such as zinc, copper, the herbicide bentazon, the herbicide degradation product 3,4-dichloroanilin, and the fungicide tebuconazole. The results suggest that the TOX-MOB assay is an ecologically relevant toxicity assay, and that methane oxidizing bacteria in soils are potentially adversely affected by compounds commonly used in modern agriculture.

## **2.16.P-Mo146 Initial Soil Microbial Colonizers Following Fresh Crude Oil Spill Incident**

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Crude oil pollution is an ecological perturbation that disrupts the biodiversity of the affected environments. This study investigated the availability of microbial isolates capable of withstanding the change in physicochemical parameters occasioned by the spill. The study also sought to identify those isolates capable of early colonization and utilization of the crude oil pollutant shortly after the spill at the 12<sup>th</sup> Santa Barbara spill site, a wetland, in Nembe Local Government Area (L.G.A) of Bayelsa State, Nigeria (4°31'60" North, 6°16'60" East). Physicochemical parameters were determined according to regulatory protocols in Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN). Microbial isolates were isolated using the enrichment technique and tested for crude oil degradation abilities using the redox-dye (2,6-Dichlorophenolindophenol) protocol. The high performing degraders were then identified using the 16S rRNA Sanger sequencing method and phylogenetic tree constructed to determine species relatedness. The mean extractable total petroleum hydrocarbons (TPH) content of 17 000 mg/kg, which is above the 5000 mg/kg intervention limit of the Nigerian Upstream Petroleum Regulatory Commission (NUPRC). The site also had an average pH of 6 and heavy metals levels within acceptable limits. The high degraders identified in the study included mainly species of *Pseudomonas* and *Acinetobacter* which are typical crude oil polluted sites microflora and presumably novel bacterial genera. This preliminary study suggests adequate presence and richness of microbial isolates for *in situ* bioremediation given the remoteness and sensitivity of the spill site.

## **2.16.P-Mo147 Microbial Indicators of Stress in Crude Oil-Impacted Soils**

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Soil environments impacted by crude oil pollution adversely affect ecological functions in such environments. The use of microorganisms especially bacteria afford an easy and timely understanding of the pollutant effects on soil biodiversity. Crude oil spill is a frequent occurrence in the Niger Delta region of Nigeria and some spills have been left unattended hence constituting chronic exposure of the resident microflora. For this study, physicochemical parameters were analyzed to determine the concentration of extractable total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) in the soil. Shotgun/whole genome sequencing from ZymoBIOMICS services was used to determine the identity and functionality of the resident microflora in the soil microbiome. Using the OMICS dashboard of the BIOCYC, pathways were reconstructed and the significant stress related genes included genes for starvation, heat shock, DNA damage, osmotic stress, oxidant detoxification, and pH tolerance. The findings show strong correlation between pollutant presence and increase in microbial stress genes.

#### 2.16.P-Mo148 Whole Genome Sequencing Reveals a Wide Range of Virulence and Antibiotic Resistance Determinants in Oil-Polluted Soil Microbiome

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Crude oil pollution is an endemic environmental problem in the Niger Delta Region, Nigeria, and as such impacted soil ecosystems over the years have been subjected to colossal biodiversity crises. The emergence of antimicrobial resistance (AMR) and virulence factors in oil-polluted soil microbiome has really not been a major research focus in this region. Following increasing evidence-based reports from our recent investigations of the worrisome presence of many AMR genes in hydrocarbon-degrading bacteria (HDB), the present study employed high-throughput sequencing technique to evaluate the diversity of these genes in the environment. Axenic cultures of HDB from the soil contaminated with 4,923 – 51,780 mg/kg of total petroleum hydrocarbons (TPH) were repeatedly screened by enrichment in 1% crude oil- and naphthalene-Bushnell Haas (BH) broth and isolates with enhanced degradation potentials were selected on BH agar. DNA extraction was performed using ZymoBIOMICS-96 MagBead DNA kit while Nexera DNA flex library prep kit was used to prepare sequencing libraries. Raw sequence reads were trimmed to remove low quality fractions and adapters. Antimicrobial resistance and virulence factor gene identification were performed with Diamond sequence aligner. Genome analysis by composition barplots at strain level revealed the presence of *Pseudomonas nitroreducens* (42.52%), *Ochrobactrum intermedium* (37.7%), *O. intermedium* 2745-2 (19%), *O. intermedium* M86 (0.5%), *Pseudomonas* sp. (0.3%), and *P. nitroreducens* NBRC 12694 (0.2%). Thirty seven antibiotic resistance genes affiliated more with *Ochrobactrum intermedium* than other strains with aminoglycoside 2'-N-acetyltransferase AAC (2')-IIa gene having read counts of 40 as the predominant determinant followed by class C extended spectrum beta-lactamase OCH-1 with 37 read counts. *Pseudomonas nitroreducens* had 5 different AMR genes that were mainly multidrug efflux transporter permease subunit complexes with read counts of 1 to 4. Virulence factors detected were 1,239 and all affiliated with *Ochrobactrum intermedium* (predominant one being glutamate synthase NADPH large chain with 1,031 read counts) and *Pseudomonas nitroreducens* (that had 'still frameshift probable component of chemostatic signal transduction system' with 405 read counts). Consequently, we conclude that oil-impacted soil ecosystems in this economic haven may possibly pose serious public health risks as important reservoirs of AMR genes and virulence factors.

#### 2.16.P-Mo149 Could Nature Successfully Trigger Soil Functional Processes in Metal Mine Tailings or Do We Always Need a Manmade Restoration Intervention?

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Metal mine wastes from ore processing are among the most hazardous residues (e.g., high metal(loid) levels, extreme pH, high salinity, organic matter/nutrients deficiency). These wastes are often deposited in open-air piles (mine tailings). Classic-restoration approaches (technical reclamation) usually involve topsoil capping aiming to create a "new soil" above wastes and then afforestation. These options have been questioned in (semi)arid regions due to ecosystems inability to be self-sustaining. An alternative, based on nature-based solutions principles, is to promote the growth of plants directly in mine wastes (phytomanagement by phytostabilization). This contributes to create the so-called fertility islands that may promote plant-soil feedbacks and ecosystem functioning. If tailings pose immediate risks (e.g., structural collapse, close to urban areas), the necessary financial/technical means should be made available for immediate technical restoration. However, if conditions are not so extreme, the spontaneous succession of plants directly growing in mine wastes has some advantages (e.g., plants adaptation to local climate/substrate conditions, high natural value, improved long-term sustainability). Previous works showed that the direct growth of native plants in tailings can provide functional soil-vegetation systems. This study aimed to evaluate how and to what extent technically reclaimed (capping and afforestation) and spontaneously colonized metal(loid) mine tailings soils differ in functional aspects. For this, a complete seasonal monitoring program (physical, chemical, physicochemical, and biological soil parameters) was carried out during 2022 in two mine tailings from SE Spain: a tailing technically reclaimed by capping and afforestation, and a tailing spontaneously colonized by plants directly growing in mine wastes. Both tailings store similar type of mine wastes and were restored/abandoned at the same time. In each tailing monitoring plots were established in areas with both shrub-herbaceous and tree vegetation. Among others, in each study season, a battery of biological parameters (in)directly related to soil functionality was measured: respiration (in situ soil CO<sub>2</sub> emission), fauna feeding activity (bait lamina), microbial biomass carbon/nitrogen, dehydrogenase activity, enzymes activity related to soil biogeochemical cycles (e.g.,  $\beta$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase, protease, urease, acid phosphatase).

## 2.16.P-Mo150 Bioconversion of Olive Pomace by *Hermetia illucens* Larvae to an Organic Fertilizer - The Soil Health and Safety Perspective

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Bioconversion of various organic wastes by black soldier fly (BSF) larvae can be an alternative method instead of conventional organic waste treatments and disposal. *Hermetia illucens* is an insect with high protein and fat content, a short lifecycle, and high bioconversion rates. Thanks to these properties, it can play a crucial role in waste management. Organic waste, such as olive pomace, which has phytotoxic characteristics mainly due to phenolic compounds, is an obstacle for the olive oil industry in terms of waste management. Bioconversion of olive pomace by BSF larvae can be a suitable alternative, while olive pomace can feed BSF. However, the generated frass from BSF's metabolic activities during the biodigestion (larvae faeces, exuviae, and organic processed material) has been widely studied and successfully applied as an organic fertilizer. To the best of our knowledge, this is the first work that assessed the frass from the biodigestion of olive pomace. The study aimed to evaluate the safety aspect of BSF frass produced from the biodigestion of olive pomace as an organic fertilizer, using soil health and quality indicators. The effects on soil organisms were assessed by incorporating frass into the natural LUFA 2.2 soil at six application rates (from 0.3 to 9.6 %w/w) and two distinct incubation periods (2 and 32 days of soil incubation with OF), conducting ecotoxicological assays with terrestrial invertebrates (OECD reproduction assays with *Folsomia candida* and *Enchytraeus crypticus*) and plants (phytotoxicity bioassays with *Lolium perenne* and *Brassica oleracea*). Plant experiments were based on two methodologies, confirming seedlings' emergence and growth in soil and aqueous extracts. Soil enzymatic activity was assessed at the same application rates of frass and three incubation periods (2, 32, and 64 days). Results demonstrated no adverse effect on soil invertebrates' survival and reproduction. Phytotoxicity tests indicated a significant increase in the germination index of *L. perenne* on day 32 of incubation compared with day 2. Overall, enzyme activity stimulation was observed by increasing the concentration of frass applied in the soil (starting from 2.4, or 4.6 % w/w). While this study provided a comprehensive evaluation of the safety aspect of bioconverted olive waste by BSF to organic fertilizer, more in-depth understanding is still required before environmentally safe and sustainable applications of the frass.

## 2.16.P-Mo151 Collembola Locomotion Behavior Test: An Alternative Method to Assess the Impacts of Pesticides on Soil Collembolans

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In soil ecotoxicology, the effects of pesticides on nontarget soil invertebrates are mainly assessed by standardized protocols of acute and chronic toxicity tests. Although the standard assays are practical and useful for ecological risk assessment approaches, they do not address the impacts of pollutants on the locomotion of organisms. The ability to move is essential for collembolans to search for food, avoid predators, and reproduce. In this work, we propose a novel method to assess the potential impacts of pesticides on the locomotion behavior of collembolans *Folsomia candida*. Initially, collembolans were exposed to an Entisol artificially contaminated with a commercial formulation of the insecticide imidacloprid. The soil was spiked with a contaminated aqueous solution to achieve a final concentration of 0.1 mg active ingredient kg<sup>-1</sup> dry soil. A control treatment containing only distilled water was also prepared. The control and the contaminated soil had their moistures adjusted to 60% of the soil water holding capacity. Ten collembolans (20-22 days old) were inserted in each experimental unit. Three replicates were prepared for each treatment. Collembolans were fed with *Saccharomyces cerevisiae*. After seven days of exposure, the soil of each replica was flooded, and collembolans were gently transferred to a clean culture plate (charcoal/plaster of Paris substrate), where they remained for 24 h to acclimatize. Then, 10 collembolans randomly selected from each group had their locomotion recorded in a video for one minute. For this, the collembolans were individually transferred to a new culture plate in which a high-resolution camera recorded their movements. The videos were analyzed in the software Kinovea® to calculate the total walked distance. The mean walked distance (n = 10) of the exposed group was compared to the control group through Tukey's HSD test (p < 0.05). We observed that collembolans exposed to 0.1 mg kg<sup>-1</sup> had reduced locomotion (p = 0.00045) compared to the nonexposed ones. A 31% reduction in the walked distance was found when comparing the mean distance of the control (19.9 ± 4.5 cm) with the contaminated group (13.7 ± 4.9 cm). The decrease in the collembolans' locomotion is probably due to the neurotoxic nature of imidacloprid, which is known to cause neuronal overexcitement and paralysis in exposed organisms. The results confirm the new method's effectiveness as an alternative to the standard avoidance behavior test.

## 2.16.P-Mo152 Rare Earth Elements (REE) Toxicity on Soil Invertebrate's Population and Microbial Decomposition in West African Soil

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Rare earth elements (REE) are metals crucial to the green economy because they are used for several commercial purposes. Global REE production accounted for around 280,000 metric tons of rare earth oxides in 2021, and Africa has a significant deposit of rare earth elements. However, very few studies have reported the toxicity of REE to soil fauna and ecological processes in African soil. Therefore, this study reported the toxicity of three REEs; Lanthanum (La), Neodymium (Nd), and Niobium (Nb),

including Nickel (Ni) as a model industrial metal on soil invertebrates and ecological processes. Here, we exposed the soil oribatid mites, *Oppia nitens* and collembola, *Folsomia candida* to these metals in three West African soils from Nigeria and the Republic of Benin. We also measured soil microbial decomposition via a filter paper experiment. As a result, these REEs reduced the mite and collembola population across the three soils and also caused a significant reduction in microbial decomposition. This study's result provides one of the first sets of data on REE toxicity to soil invertebrates in West African soil. It will set the stage for soil ecological risk assessment of REE in West Africa.

#### **2.16.P-Mo153 Impact of Initial Body Weight of *Eisenia fetida* on Main Parameters Mortality, Body Weight Change, and Reproduction in OECD 222 Earthworm Reproduction Testing**

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OECD guidelines for environmental risk assessment (ERA) of plant protection products for nontarget soil organisms have been published to meet criteria such as reproducibility and comparability. In order to increase standardisation and to reduce variability in the data sets, the test organisms should meet certain criteria. These criteria include certain life stage(s), age, or other important life history parameters such as sexual maturity or body weight with respect to earthworms in OECD 222 (2016) tests. For the earthworm *Eisenia fetida*, the initial weight of animals used in these tests should be between 300 and 600 mg.

The main objective of this poster is the assessment if this range is appropriate or if the initial weight of the adult earthworms have an impact on the main parameters (mortality, body weight change, and reproduction) that need to be determined in the tests. To test this, an OECD 222 test was run in untreated artificial soil using “light” (300-350 mg) and “heavy” (550-600 mg) mature worms. In a second step, the impact of initial body weight on toxicity of the reference item boric acid to *E. fetida* was investigated using “light” and “heavy” mature worms. Adult mortality and body weight change were determined after 28 days and the number of juveniles was assessed after 56 days. The results are presented and discussed in the light of standardisation and ERA.

#### **2.16.P-Mo154 Single and Combined Effects of Metal-Based Fungicides on *Eisenia fetida* under Different Climatic Change Scenarios**

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This study evaluated the ecotoxicity of metal-based fungicides under the current global change scenarios. It sought to determine the response of *Eisenia fetida* to copper oxychloride (CuOx) and mancozeb (MnZn) under varying temperatures (20°C and 25°C) and soil moisture content regimes (30% and 50%). The concentrations for CuOx were 200, 500, and 1000 mg/kg and MnZn, 44, 850, and 1250 mg/kg in single and binary mixtures. The test assessed mortality, growth, avoidance behaviour, and reproduction as biological endpoints following standardized protocols (ISO and OECD). The mortality rate in all single-exposure groups was below 10% in all the treatment conditions except CuOx1000. In the mixture exposure groups, the mortality rate only exceeded 10% in the CuOx1000 and CuOx1000 + MnZn1250 mg/kg groups in all the temperature-moisture combinations. However, the mortality rate exceeded 10% only in the CuOx500 + MnZn850 mg/kg treatment at 20°C30%. In the CuOx and MnZn treatment groups, the relative growth rates decreased with increasing concentration. In both CuOx and MnZn treatment concentrations at 20°C30% and 25°C50% conditions, avoidance response behaviour was above 80% throughout the 48-h exposure except in the CuOx200 mg/kg, MnZn44 mg/kg and the binary mixture of CuOx200+MnZn44 mg/kg. The reproduction of the exposed earthworms across all treatment groups was concentration-dependent and influenced by the varying temperatures and soil moisture conditions. There were no juveniles or cocoons in the CuOx1000 mg/kg treatment at 25°C30%, thus indicating that copper oxychloride may be more toxic than mancozeb, especially in drought conditions (increased temperature and reduced moisture). The binary mixture of CuOx and MnZn had no synergistic effect across all treatment conditions. This study found that different temperature and soil moisture level altered the ecotoxicity of CuOx and MnZn. Therefore, climate change is likely to significantly impact the outcomes of metal ecotoxicity to earthworms and their ecological activities.

#### **2.16.P-Mo155 Effect of Macroplastic on Soil Percolation and Infiltration Rate: A Lysimeter Test using Artificial Rainfall on Samples from the Central Norwegian Coast**

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Mausundvær is a group of remote, protected islands located along the outer coast of central Norway, where large amounts of ocean plastic waste are known to accumulate due to a bend in the North Atlantic Current. Previous studies have shown that soil accumulating just above the upper tide level on Mausundvær contain up to 70% (dw/dw) of plastic, with a range down to approximately 10%. In this study, core-samples will be taken from selected areas with a broad range of plastic concentrations. Each sediment core will then be exposed to an artificial rain experiment and percolation tested using a lysimeter in a laboratory setting. Finally, all samples will be dried and the concentration of macroplastics quantified for each core. Expected results will document the impacts of macroplastic concentration on infiltration and percolation of water through organic-rich coastal soils, and on drying rates. Results will be discussed in relation to existing research on how plastic might affect the decomposition of organic matter and therefore the carbon cycle. A critical discussion of methods and protocols will also be presented.

#### **2.16.P-Mo156 The Resilience of Soil Systems Toward Microplastics**

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The impact of microplastics (MPs) on soil health and fertility has been a hot topic for the last couple of years. However, the effect of MPs on soil resilience has not yet been investigated, particularly when comparing the effects of different MP materials. A threshold of resilience is needed to improve our understanding of how MPs influence soil resilience. We hypothesize that MP exposure influences soil properties and functioning, and accumulation will negatively impact the resilience of soil systems. In this study, we are performing a laboratory experiment using varying concentrations of MPs in soils and measuring changes in bulk density, porosity, organic matter, pH, and water-holding capacity of soils. These changes will be tracked across several time intervals over several months to understand how soils respond and potentially recover from the addition of microplastics as a disturbance. The ratio of postdisturbance and predisturbance will be considered to evaluate soil resilience. This will include the development of approaches to evaluate soil resilience in the context of persistent and particulate contamination. The preliminary results of the experiment will be presented as a poster during the SETAC conference.

## 2.16.V Soil Function and Biodiversity: Impacts and Resilience Under Stressed Environments

### 2.16.V-01 Ag<sub>2</sub>S NP Accumulation in the Mealworm *Tenebrio molitor* and the woodlouse *Porcellio scaber*: Are Single-Species Tests a Good Predictor of Indoor Mesocosm Experiments?

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Silver nanoparticles (Ag NPs) are used in everyday applications, ending up in wastewater treatment plants (WWTPs) and accumulating in sewage sludge. Because of the high sulfide concentration in WWTPs, AgNPs are likely transformed into Ag<sub>2</sub>S. Upon application of biosolids onto agricultural land, as fertilizer, exposure of terrestrial organisms to Ag<sub>2</sub>S is inevitable. The objective of this study was to validate the prediction of toxicokinetics of Ag in invertebrates exposed to Ag<sub>2</sub>S in multispecies indoor mesocosm experiments from earlier single-species tests (Khodaparast et al., 2021; Talaber et al., 2020). The mealworm *Tenebrio molitor* and the woodlouse *Porcellio scaber* were exposed in mesocosms together with plants (*Triticum aestivum* L.) and earthworms (*Lumbricus rubellus*). The organisms were kept for 28 days in soil columns which contained a 10 cm top layer spiked with 10 µg Ag g<sup>-1</sup> dry soil as Ag<sub>2</sub>S NPs or AgNO<sub>3</sub> and a bottom layer of clean soil. The soil columns were watered daily with artificial rainwater. The organisms were collected at different sampling times (0, 7, 14, and 28 days), purged for 24 h, and then frozen, dried, and acid digested to measure the total Ag concentration. The soil pore water was also extracted to measure total and dissolved Ag concentrations. A one-compartment model was used to estimate the Ag uptake kinetics. Although Ag<sub>2</sub>S NPs dissolved much less than AgNO<sub>3</sub>, the dissolution of Ag<sub>2</sub>S NPs in the soil pore water extracted from the mesocosm test was higher than during the single-species test. In the case of mealworms, the uptake rate constants from AgNO<sub>3</sub> in both tests, single-species and mesocosm experiments, were similar regardless of exposure time. But Ag from Ag<sub>2</sub>S NPs was taken up faster in mealworms during the mesocosm experiment compared to single-species tests. For woodlice, the uptake rate constant of Ag from AgNO<sub>3</sub> in single-species tests was significantly slower than in mesocosm tests and woodlice did not bioaccumulate Ag from Ag<sub>2</sub>S NPs in both single-species and mesocosm tests. To conclude, the single-species tests may not be an accurate predictor for the Ag uptake in mealworms and woodlice under more realistic exposure conditions of a mesocosm. But this does not mean that single-species tests could not be used as a fast screening approach to assess the possible bioaccumulation of chemicals in biota before other more complex tests can be conducted.

### 2.16.V-02 Earthworms under Plastic Debris and Environmental Stress. The Case of Agricultural Microplastics

**Esperanza Huerta Lwanga<sup>1</sup>**, Dana Bashkir<sup>2</sup>, Paul Domin<sup>1</sup>, Paolina Garbeva<sup>3</sup>, Alice Wang<sup>1</sup>, Giulia Bonjorno<sup>1</sup>, Violette Geissen<sup>1</sup> and Coen Ritsema<sup>1</sup>, (1)Wageningen University & Research, Netherlands, (2)Koblenz University, Germany, (3)NIOO-KNAW Earthworms, known as soil ecosystem engineers and soil health indicators, are in danger when soil emergent pollutants are present. In agricultural systems microplastics are found in the soil when plastic mulches are used, or when compost is applied. Although it is known how high concentrations of microplastics impact earthworm abundance and diversity, scarce information is provided when extreme environmental conditions are present. Therefore, the aim of this study was to evaluate the performance of earthworms under extreme environmental conditions with and without microplastics and with and without plants.

More than 100 experiments were performed with 3 soil types, 3 plastic types, and 3 earthworms' ecological categories. We observed how with low microplastics concentrations environmental conditions play a key role. Preliminary results indicate how anecic and epigeic earthworms are the most vulnerable, and endogeic earthworms are the most resilient ones, and multispecies were also very resistant. Plant performance seems to be dependent on environmental conditions and earthworms' performance. More results will be later provided.

## 2.17.P Toward a Better Understanding of Chemical Biomarker Responses in Aquatic Organisms

## 2.17.P-Tu119 Living under CO<sub>2</sub> Pressure: Investigation of Some Oxidative Stress Enzymes and Metabolic Markers in Two Calcifying Mollusks from the CO<sub>2</sub> Vent of Ischia Island (Italy)

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The effects of ocean acidification (OA) are becoming one of the main investigative targets for understanding the anthropogenic impacts on the marine environment. Particularly, we focused on naturally acidified systems, such as the CO<sub>2</sub> vents at Ischia Island (Italy), as a natural laboratory to study the long-term effects of OA on two calcifying organisms: *Patella* spp., which are long-time residents in the vents systems, and *Mytilus galloprovincialis*, that occur only as juveniles in the vents. Our aim was to understand the potential mechanisms underpinning the tolerance processes occurring in response to OA in these calcifying taxa.

We investigated the activity of electron transport system and the glycogen content as parameters related to metabolic capacity, the activity some antioxidant enzymes (CAT, SOD, GST, and GPx), the oxidative damage such as lipid peroxidation, and the extent of valves decalcification. Our results show that limpets seem adapted to OA, and, in these organisms, low pH conditions do not trigger significant oxidative stress; on the contrary, mussels show strong decalcification of valves, alteration of the antioxidant system and a reduction of glycogen content. This suggests that early stages of acclimation to OA might involve the imbalance of osmoregulation and a significant energy consumption in order to survive at low pH.

## 2.17.P-Tu120 Pinpointing the Effects of Oceanic Pollutants in Blue Sharks (*Prionace glauca*) with High-Throughput Transcriptomics

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The blue shark (*Prionace glauca*) is a cosmopolitan species, being one of the most widely distributed and heavily caught pelagic shark species across the globe. As a top predator with a long life span, this species is very prone to accumulate high levels of oceanic pollutants through bioaccumulation and biomagnification processes. The detoxification of most pollutants is performed in their liver, which is one of the largest organs in these species. With the use of transcriptomics methodologies for whole gene expression profiling, a more global assessment of the health status of an organism can be achieved through an understanding of the functional pathways that are responding to pollutant exposure. Thus, this study aimed to assess the influence of different contaminants' body burden (metals and POPs) in the liver transcriptional profiling of *P. glauca*, for an improved understanding of the organisms' mechanisms to cope with different ocean contamination scenarios. As very little genomic information was available for *P. glauca*, and to improve quality and coverage of the gene expression profiling results, the genome of the species was for the first time assembled and annotated using PacBio HiFi data and both long- and short-read RNA sequencing data from different tissues. To achieve the goal of this study, RNA from the liver of 21 juvenile male blue sharks caught as bycatch in swordfishing vessels was sequenced and mapped against the assembled genome. The transcriptional profiles were then analysed against the contaminant levels measured in the same tissue following a multivariate correlational analysis. The top 120 correlated genes were selected and clustered. A GO enrichment analysis of each cluster demonstrated lipid and fatty acid metabolism to be positively correlated to metal levels and several regulatory mechanisms negatively correlated to these elements. Sharks are believed to possess an extremely adaptive immune system, and a general positive correlation between immune system related genes (e.g., immunoglobulins) and POPs levels was also highlighted in this study. This study underlines the relevance of 'omics data to better understand the overall effects of contamination in real exposure scenarios and the importance of similar studies for monitoring the marine environment. Furthermore, the blue shark genome will be a valuable resource and expand the capacity for in-depth molecular investigation on elasmobranchs, shark immunity, and vertebrate evolution.

## 2.17.P-Tu121 Fish Scale Hormone Concentrations as a Nonlethal Biomonitoring Tool for the Assessment of Chronic Stress and Endocrine Disruption

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Fish serve as an essential resource to the human population and thus their conservation is of extreme importance. Nonlethal biomonitoring tools are invaluable in the accomplishment of such goals as they allow for the collection of information without adversely impacting populations at risk. Within the past decade the use of fish scale cortisol concentration as a nonlethal method for the assessment of chronic stress in teleost fishes has been explored. This has been highly successful with scale cortisol concentration now shown to increase in response to a variety of chronic stressors such as increased water temperatures, physical stressors, and fin injury. However, alongside cortisol the stress response is mediated by additional steroid hormones. This can include DHEA, an androgen and precursor steroid capable of influencing the conversion of cortisol to the inactive metabolite cortisone. Therefore, to expand upon this approach we quantified cortisol, cortisone, and DHEA in control and stressed rainbow trout (*Oncorhynchus mykiss*) scales and serum. Increases in cortisol, cortisone, and DHEA observed in stressed trout scales were not reflected in serum samples, adding evidence to the practicality of scale hormone concentrations in chronic stress assessment. In a second study we successfully quantified scale progesterone, testosterone, and 11-ketotestosterone alongside cortisol in rainbow trout to allow for the additional assessment of alterations to reproductive endocrinology. This study identified negative relationships between scale cortisol and testosterone, a relationship often used to quantify the adverse effects of stress on reproduction. The addition of reproductive steroids to our repertoire also presents an opportunity for future identification of other sources of endocrine disruption in fishes such as that induced by chemical contaminants. Thus, while these methods are still under

development, our results provide promising evidence of the practicality of fish scale hormone concentrations for the nonlethal biomonitoring of teleost fishes.

### **2.17.P-Tu122 Diatom Role in River Ecological Assessment: Linking Diatom Diversity Determined by Molecular Methods to Some Environmental Features**

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Anthropogenic pollution is deemed as the primary source of stressors threatening freshwater aquatic ecosystems. The extensive use of fertilisers, the release of wastewater, and the artificial alteration of the surrounding land can be reflected in the physicochemical characteristics of riverine water. In turn, this can have adverse effects on the biotic communities like diatom assemblages. These organisms are considered valuable bioindicators due to their swift response to environmental changes displayed in their assemblage composition. The importance of this information lies in understanding the impact imposed by stressors on the river quality.

The WAT-DIMON project aims at the creation of a novel genomic test for identifying European benthic diatoms through DNA metabarcoding techniques which can improve and expediate freshwater bioassessment. Extracted results can then be compared with the ambient physicochemical parameters, aiming at the evaluation of the environmental impact of stressors and early treatment response.

For this work, epilithic diatom samples have been collected from rivers at 52 sampling sites across four biogeographical regions in Europe, and their physicochemical parameters were measured. DNA barcodes were amplified using specific primers for diatoms and sequenced using Illumina MiSeq. Bioinformatics analysis was then performed using the DADA2 pipeline to quality-filter the sequences and identify the diatom species using the Diat.Barcode reference database. Lastly, nonmetric multidimensional scaling (NMDS) was performed to investigate the relationship between diatom assemblages and river characteristics.

The preliminary results show a variation in the diversity between the river samples. Furthermore, the NMDS ordination revealed dissimilarity between the diatom assemblages of the different biogeographical regions. The river type (Intermittent – periodically cease flow and Perennial – continuous flow) and the pH also seemed to influence the diatom assemblages. Even though these results suggest an influence of the physicochemical parameters on the diatom assemblages, they are nonconclusive and will be further analysed.

### **2.17.P-Tu123 Using a Translocation Experiment with Feral Brown Trout to Investigate Anthropogenic Impacts on Fish**

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Water bodies are impacted by a constantly growing number of pollutants that are present in complex mixtures. The ecological effects of exposure to these mixtures are most often difficult to assess, as impact-oriented *in situ* studies that take realistic scenarios into account are scarce.

This study aims at investigating the impacts of multiple chemical stressors on feral fish by analysing biomarker and transcriptomic response patterns in locally adapted and translocated individuals. Complemented by comprehensive chemical analysis, this study will strengthen the understanding of the causal relationships between environmental pollution and ecological impacts.

At three sites, following a gradient of anthropogenic influence along a well-studied small river (Holtemme) in Central Germany, feral brown trout (*Salmo trutta fario*) were caught with hand-held electrofishing gear and caged at the same locations.

Furthermore, fish from a reference site were relocated and caged at two sites upstream and downstream of the wastewater treatment plant (WWTP) Silstedt. Each of the five groups comprised 24 fish distributed in three cages.

After 21 days of exposure, fish were euthanised, exsanguinated and organs were dissected. Tissue and blood samples will be analysed using a broad battery of well-established enzymatic and non-enzymatic biomarkers indicative of oxidative stress, xenobiotic metabolism, immune response and neurological disturbance.

Furthermore, transcriptomic patterns will be investigated by next-generation sequencing to identify underlying molecular pathways of physiological response processes.

Simultaneous with the caging, time-integrated water samples were taken using Large-Volume Solid Phase Extraction at each sampling site. These samples undergo targeted high-resolution LC-MS and bioanalytical analysis to identify compounds that drive toxicity.

Preliminary results show that fish caged downstream of the WWTP exhibited higher mortality during exposure time than at the other two sites. The liver somatic index was significantly lower in the group of fish relocated from the reference to the downstream site, which might indicate elevated energy consumption during the experiment.

#### **2.17.P-Tu124 Can sub-lethal effects of plastic ingestion be assessed by phthalates concentration and molecular biomarkers in stranded Manx shearwaters, *Puffinus puffinus*?**

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Seabirds are considered sentinels of marine ecosystems as they reflect environmental changes. Plastic pollution is one of the greatest challenges for marine conservation, and nearly all living seabirds will ingest plastics at some point in their life. Additives (e.g. phthalate esters) can leach from plastics and because seabirds often retain plastic items for extended periods in their digestive tract, they could be vulnerable to the adverse effects of chronic exposure to these compounds. In this study we evaluated molecular biomarkers in stranded Manx shearwaters (*Puffinus puffinus*) as a tool to assess sub-lethal effects of plastic ingestion. Age class, sex, body mass, and plastic presence were registered for all birds. The transcript levels of genes involved in xenobiotic biotransformation including aryl hydrocarbon receptor (*AhR*), cytochrome P450 1A5-like (*CYP1A5*) and UDP-glucuronosyl-transferase (*UGT1*); genes related to endocrine disruption as estrogen receptor alpha (*ESR1*); and genes responding to metabolic stress as heat shock protein 70 (*HSP70*) were measured in the liver from 79 fresh carcasses of birds (11% with plastic) stranded along the coast of southern Brazil from 2016 to 2021. Plastic additives (Dibutyl phthalate - DBP, Dimethyl phthalate – DMP, and Diethylhexyl phthalate - DEHP) were also quantified by gas chromatography with flame ionization in the uropygial gland from 25 birds (8% with plastic). Overall, 11 of the 104 seabirds sampled had ingested plastic found during necropsy. Of these 11 shearwaters, 55% were males and all were juveniles. The best-fitting generalized additive models (GAM) found associations between the transcript levels of *AhR* and *UGT1* genes responding to bird age class, sex, mass, and plastic presence. Slightly higher transcript levels of both genes in animals with plastic showed a potential positive association. Additives were detected in 10 seabirds for DBP, 2 seabirds for DMP, and 12 for DEHP. GAMs best fitting models showed associations between DEHP concentration and the factors sex and plastic presence. Although preliminary, our findings imply the need to further investigate sublethal effects of plastics, as well as their influence on birds' metabolism and response to environmental xenobiotics. This study provides baseline data for the conservation of seabirds in an area where plastic pollution is increasing.

#### **2.17.P-Tu125 Isotopic variation in response to the combined chemical exposure and temperature**

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Stable isotope signatures for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) are widely used in trophic ecology and ecotoxicology for the trophic positioning of consumers. The approach relies on 'predictable' isotope fractionation in the consumer tissues relative to their diet, with the physiological condition of the animals having a relatively low effect on the fractionation. However, when chemical and nonchemical stressors affect the physiological status, these signatures may also be affected due to responses in biochemical rates and altered pathways, with downstream effects on fractionation.

Understanding the effects of various stressors, such as chemical pollution and temperature, on the isotopic signatures is essential for their interpretation. Departing from the premise that sublethal chemical exposure distorts fractionation and isotopic signatures via altering metabolic rate, we aimed to assess stable isotope variation as an indicator of toxic effects under different thermal conditions. Here, using a passive dosing system with no food, we exposed *Daphnia magna* to the combined effects of polycyclic aromatic hydrocarbons (PAHs; acenaphthene, fluorene, phenanthrene, and fluoranthene as a mixture) and temperature (20°C, 23°C, and 25°C). We used the chemical activity concept to address the effects of the PAH mixture and relate the chemical exposure and temperature effects to the metabolic alterations in the daphnids. We measured  $\delta^{13}\text{C}$  profiles, mortality and protein content and established their dose-response relationships ( $Ea_{50}$ ) for different chemical activities in the range of 0.01 to 0.1 and the three thermal conditions.

At all temperatures, a depletion of  $\delta^{13}\text{C}$  was observed with increasing chemical activity in a dose-response fashion. Moreover, the  $Ea_{50}$  values were temperature-dependent, decreasing from 0.08 at 20°C to 0.05 at 23°C and 0.06 at 25°C. In addition, low protein content and high mortality coincided with low  $\delta^{13}\text{C}$  values, supporting the view that isotopic fractionation is affected by metabolic depression. Understanding non-dietary isotopic variability in a multi-stressor context improves the interpretation of stable isotope signatures in environmental assessment.

#### **2.17.P-Tu126 Fatty acid profiles in blubber samples from humpback whales (*Megaptera novaeangliae*) between feeding (Southern Ocean) and breeding and calving areas (NE Brazil)**

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Humpback whales (*Megaptera novaeangliae*) in the southern western hemisphere feed during summer and autumn in the Southern Ocean and the southern coast of Chile and migrate northwards to mate and reproduce in northeast region of Brazil (known as Stock A) and in the Caribbean region (Stock G). The energy expended during this displacement is enormous and cause a loss of approximately 20-40% of their blubber layer, since the humpbacks are fasting from the calving and breeding areas until their returns to feeding sites. In order to evaluate possible changes on the superficial blubber layer composition, samples from stranded whales (n=20) in Brazilian Coast and biopsies (n=81) from specimens around Antarctic Peninsula were collected to assess fatty acids. Results indicated the predominance of similar fatty acids in the samples collected in the breeding and feeding areas, with highest concentrations of monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), with C18:1 $\omega$ 9, C16:0, C16:1 $\omega$ 7, C14:0 and C18:1 $\omega$ 7 as the compounds with the highest contributions. Samples from the northeastern and southeastern Brazilian coast presented a relatively higher contribution of long-chain polyunsaturated fatty acids (PUFA), such as C22:5 $\omega$ 3 (DPA), C22:6 $\omega$ 3 (DHA) and C20:5 $\omega$ 3 (EPA) compared to Antarctic samples, even though the average EPA/DHA ratio was quite similar to krill (*Euphausia superba*), the main prey for the humpback whales sampled in this study. The fatty acid profile differences can be directly related to the metabolization of the lipid layer during the feeding and fasting period although individual aspects such as gender and age should be considered to better understand how this highly migratory species adapts during migration.

## **2.17.P-Tu127 Linking Hepatic Residues of Anticoagulant Rodenticides in Wild Freshwater Fish with in vivo Determined Effect Levels**

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Second-generation anticoagulant rodenticides (SGARs) remain the most often used biocides to control pest rodents such as house mice or brown rats despite bearing high risks of unintentional poisoning of wildlife and being classified as persistent, bioaccumulative and toxic (PBT). Consequently, these compounds will continue to be detected regularly in an ever-increasing number of non-target organisms. While primary and secondary poisoning of terrestrial and avian species such as small mammals and raptors are well known, little data exists on fate of SGARs in the aquatic environment and to an even lesser extent regarding possible ecotoxicological effects associated with this exposure levels. Although recent studies found AR residues in livers of freshwater fish in the magnitude of ng/g, it is currently not possible to link these hepatic AR concentrations to possible lethal or sub-lethal effects.

In order to determine the relevance of reported SGAR residues in wild freshwater fish and to assess their risk of AR poisoning, we conducted *in vivo* experiments feeding rainbow trout (*Oncorhynchus mykiss*) defined doses of the SGAR brodifacoum in a flow through system. In a first experiment, one-year old fish (ca. 250 g) were exposed individually to brodifacoum-spiked feed for 15 d whereas in a second experiment one and a half year old trout (ca. 650 g) were exposed in groups to the SGAR for 60 d. Lower brodifacoum doses were chosen based on the limit of quantification of brodifacoum, intermediate doses based on the concentration of active ingredients in rodenticide baits and higher doses represent reported effect levels in other species. Sampling of fish every 15 d allowed tracking brodifacoum concentrations in liver, serum, and filet, and occurring effects over the course of time. Notably, the duration between brodifacoum uptake and observed effects took 15 d and was considerably longer than reported for other vertebrates. Brodifacoum reduced the vitamin K level and inhibited the coagulation in rainbow trout. Disturbed haemostasis resulted in haemorrhages in gills, pseudobranchs and other organs leading to severe anaemia and mortality. Effects on coagulation occurred at an average brodifacoum concentration of ca. 150 ng/g hepatic tissue. The results emphasize, that acute standard biotests are not suitable to observe effects of chronically acting AR on fish and would substantially underestimate potential effects.

## **2.17.P-Tu128 The Use of Comparative Transcriptomics Field Studies to Assess the Effects of Multiple Stressors on Fish**

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European running waters are affected by several multiple stressors (MTS) which can have detrimental effects on the fish fauna. In such MTS scenarios, it remains highly challenging to unravel the particular effects of a stressor on the species of interest. Furthermore, the challenge is amplified by the traditional approach of studying the effects of MTS relying on controlled small- and meso-scale experiments. In these experiments, the organisms are exposed to different levels and combinations of stressors and their effects are measured as the organism's survival or at the physiological level with a limited set of targeted physiological markers. When limiting the MTS studies to manipulative and targeted physiological studies, the effects of stressors on other physiological pathways or the effects of underlying stressors combinations in natural habitats may pass undetected. In this context, the use of comparative transcriptomics in the field has the potential to assess the effects of MTS in the entirety of an organism's gene expression, pinpoint particular stressors of interest, and even understand the mechanisms and underlying genetic pathways of the adaptation to stress. To validate the approach of comparative transcriptomics for MTS study in natural populations of fish, here we present evidence of differential gene expression in *Gasterosteus aculeatus* that correlate with different levels of anthropogenic stressor conditions in the river Boye (Germany). We chose one river transect with natural cover and mainly agricultural influx and two field sites in an urban and industrial landscape that showed higher nutrient and salinity concentrations. For each site, we did Illumina high throughput mRNA sequencing of three specimens and performed a differential



gene expression analysis. We found a set of significantly differentially expressed candidate genes between the fishes of the different transects. A gene ontology analysis of these genes showed an enrichment of gene expression in the pathways related to response to osmoregulation in both transects with higher urban and industrial land cover and higher concentrations of chloride. Following a similar research design, the transcriptome response analysis of the native and endangered fish species *Cottus rhenanus* is ongoing, and results will be presented in the poster. Our case studies showcase the comparative transcriptome approach potential to understanding fish adaptation under MTS environments in natural habitats.

#### **2.17.P-Tu129 Evaluation of physiological and morphological endpoints combined with the conventional endpoint of algal toxicity: application to five disinfection by-products characteristic of a chlorinated effluent**

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The toxicity of a contaminant present in the environment is usually assessed with standardized bioassays focusing on only one or a few specific endpoints on the organism/individual level, such as survival, growth, reproduction or development. These endpoints allow us to determine the toxicity of a specific substance on organisms, but, in that case, information on the mode of action on the subcellular level is partial or missing. In this work, the individual toxicity of five disinfection by-products (DBPs) characteristic of a chlorinated effluent (monochloroacetic acid MCAA, dichloroacetic acid DCAA, trichloroacetic acid TCAA, bromochloroacetic acid BCAA and 1,1-dichloropropan-2-one 1,1-DCP) was evaluated on the green microalgae *Raphidocelis subcapitata*. In addition to a classic growth inhibition approach (OECD TG 201), flow cytometry techniques were investigated to better understand the physiological performance of the green microalgae. Some cellular biomarkers, such as metabolic activity, cellular viability, mitochondrial membrane potential, oxidative stress, lipid content, chlorophyll *a*, green autofluorescence, size and complexity, were developed to determine sublethal responses and the mechanisms of action which could explain the effects measured on the population growth.

Results showed that 1,1-DCP, a DBP for which few information is available in the literature, was the most toxic substance for growth inhibition endpoint followed by AMCA>ATCA>ABCA>ADCA. Concerning the cellular parameters studied, all of them showed similar patterns in response to the 5 DBPs except for metabolic activity. Green autofluorescence, size, intracellular complexity, neutral lipid content and oxidative stress significantly increased during the experiment, whereas mitochondrial membrane potential showed no changes for any DBP and at any time for the five DBPs. Furthermore, a decrease in chlorophyll *a* (photosynthetic activity) was observed at 72h. In another way, metabolic activity presented some dissimilarity between compounds with a significant decrease observed after 48h of exposure to MCAA and 1,1-DCP, whereas the other DBPs led to a significant increase in metabolic activity after 48h and 72h of exposure. Based on these observations, we proposed a hypothetical comprehensive figure representing the relationships between the cellular parameters observed during the study leading to growth inhibition after exposure to DBPs.

#### **2.17.P-Tu130 The combined effect of stress and diazepam on facultative migration of European glass eel (*Anguilla anguilla*): development of endocrine and molecular markers**

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The European eel (*Anguilla anguilla*) population has received considerable attention in recent decades due to its significant decline. This catadromous species presents a facultative diadromy and glass eels display a large panel of estuarine migratory tactics, ranging from residency in marine water, to various degrees of upstream colonization through estuarine and freshwater ecosystems. These different patterns of migration can have a strong impact on the fate of the population because individuals remaining downstream mostly develop into males, whereas individuals colonizing upstream develop mainly into females. Recent results suggest that glass eels presenting a low probability to migrate in experimental facilities may be more stressed or more sensitive to stress than migrant individuals. Estuaries are considered stressful environments and represent a major sink for various contaminants usually considered as powerful stressors depending on their concentrations. Among these chemicals, benzodiazepines, found in relatively high concentrations in effluents of wastewater treatment plants, can induce a number of neuroendocrine alterations in exposed fish. However, paradoxically, they also may counteract the stress responses in fish due to their anxiolytic effect.

The aim of this work was to investigate the effect of the stress and diazepam at environmental doses on the European glass eel by developing stress markers. Stress hormones analysis were developed by LC-MS-MS to determine cortisol, cortisone, 11-deoxycortisol, and 11-deoxycorticosterone production in glass eel individuals. In addition, transcriptomic markers were detected using a non-targeted approach, by RNAseq. These molecular and endocrine markers were analyzed on glass eels contaminated or not with diazepam, and exposed or not to a 2 minutes mechanical stress. Both stress markers were monitored with time (after 15, 30, 60 and 120 min) to evaluate the combined effect of stress and diazepam in glass eel.

The developed stress markers could then be applied to address stress in migrant and non-migrant glass eels to understand the determinism of migration.

#### **2.17.P-Tu131 Co-variation of metallothionein expression levels and cadmium tolerance in *Gammarus fossarum* field populations.**

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Metallothioneins (MTs) are low molecular weight proteins rich in cysteine notably involved in the detoxification of non-essential metals. In aquatic invertebrates, MTs have been identified as potentially being involved in population adaptation to metal exposure in the contaminated environment. In one field population of the crustacean *Gammarus fossarum* naturally exposed to geochemical sources, we have previously documented an increased tolerance to cadmium (Cd) in adult organisms which can be transmitted to their offspring by means of parental effects. The objective of our study was first to generalize this result to a larger set of field populations in naturally contaminated headwater streams, and second to establish a possible correlation between *Gfoss*MTs expression and tolerance of different life stages based on organisms either freshly collected in the field or after laboratory maintenance in Cd-free conditions (6 months). For this, 11 natural populations of *G. fossarum* from Cd contaminated and uncontaminated rivers were collected after a fine *in situ* characterization of Cd bioavailability (active biomonitoring). We assessed concomitantly Cd tolerance (mortality tests) and the expression levels of *Gfoss*MTs measured by RT-qPCR in the gills and caeca of adult males and in neonates. The results show at different dates of the year, Cd tolerance during mortality tests of Cd-contaminated populations, and a clear correlation with the expression levels of one MT, namely *Gfoss*MT1 of the different batches of individuals. This covariation was also supported by a parallel decrease of tolerance and MT expression in the gills during maintenance in Cd-free water. Furthermore, a transmission of tolerance to unexposed offspring was confirmed. Interestingly, while similar levels of *Gfoss*MT1 expression were observed under control conditions in neonates born from sensitive vs tolerant genitors, we observed a higher induction of this expression in neonates from tolerant populations under Cd re-exposure in the laboratory. This pattern leads to a discussion of the plasticity mechanisms that appear to support the modification of sensitivity in exposed natural populations.

#### **2.17.P-Tu132 Ecotoxicological State Assessment of Fish and Bivalves**

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In the context of the different Marine frameworks, France implemented since 2017 an ecotoxicological monitoring on bivalves and flatfishes, namely the SELI program, on two hot spots of chemical contamination in the French Atlantic and Channel marine waters: the Loire and the Seine bays.

The present work aimed to i) develop ecotoxicological indicators for the Marine Strategy Framework Directive (MSFD) evaluation for bivalves and flatfishes (interpretation and integration/aggregation tools) and ii) integrate ecotoxicology and chemistry on different matrices (biota, sediment) on the Seine bay for the Ospar Quality Status Report (QSR).

In the Seine Bay, common sole (*Solea solea*), dab (*Limanda limanda*), and flounder (*Platichthys flesus*) were sampled during a unique campaign in September 2018. Moreover, blue mussels (*Mytilus edulis*) were collected during the annual campaign of the French mussel watch program in February 2019. In the Loire bay, two campaigns were conducted: soles were sampled in September 2017 and 2020, and mussels in February 2018 and 2021. A panel of ecotoxicological parameters, among the ICES recommendation list, were assessed in organisms. They targeted different toxic endpoints (general stress, neurotoxicity, genotoxicity, cytotoxicity) and PAH exposure (only for fishes).

The ecotoxicological integration tools allowed the evaluation of the general health status of each species and area. The results showed that flounder and mussel achieved “Good environmental status” as defined under MSFD, while sole and dab were affected by the chemical contamination.

The chemical-biological assessment conducted on the Seine Estuary allowed for the integration of the different ecotoxicological parameters, and the chemical concentrations in fish, bivalve and sediment, for several geographical units. The closest estuary unit seemed to be the most impacted with the highest percentages of both chemicals and biological effects at unacceptable levels, followed by a decrease with the distance from the coast.

These results highlighted the biological impacts of the chemical pressure in the two studied areas, particularly on fish. The sole seems to be more sensitive to pollution than the other flatfish species, putting forward the need of specific thresholds.

This work supports the need for a long-term monitoring on mobile and sessile species to allow trends assessment. Other toxicological endpoints should be investigate, such as immunotoxicity or endocrine disruption.

#### **2.17.P-Tu133 Transcriptional Biomarkers of Toxicity – An Applied Perspective from Studies on Bivalves**

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Gene transcripts may have great potential as early warning biomarkers by representing how an organism copes with chemical stress before harm arises at higher organizational levels. A major challenge is however to distinguish transcriptional stress responses from background noise and baseline variability. Using bivalves as study organisms, we assessed sensitivity, variability, and robustness in six putative transcriptional biomarkers of general stress; catalase, glutathione-S-transferase, heat shock proteins 70 and 90, metallothionein and superoxide dismutase. This was performed through a systematic review and meta-analysis on bivalves in general, and through laboratory studies on the freshwater mussel *Anodonta anatina*.

For the meta-analysis, we extracted reported transcriptional responses ( $n = 396$ ) from 22 selected studies and incorporated them into Bayesian mixed effects models to test overall responses with and without tissue, exposure concentration (transformed to toxic unit) and exposure time as moderators. In the main experimental study, *A. anatina* ( $n = 40$ ) were exposed for 96 h to a high-resolution concentration gradient of Cu ( $0.13 - 1\ 600\ \mu\text{g/L}$ ). Relative expression of selected transcripts was measured by RT-qPCR, and concentration-response relationships were fitted relative the measured Cu concentrations in both water and tissue.

According to the meta-analysis, the estimated overall response in a bivalve upon arbitrary metal exposure was an expected 65 % increase in relative expression. The effect was however inflated by publication bias toward reporting positive responses, and when corrected for bias, expected responses were close to zero. Furthermore, there was general implication of monotonic concentration-response relationships in *A. anatina* upon Cu-exposure. Modelled responses however saturated at relatively low magnitudes, and only three transcripts in gills exceeded a 2-fold change within the exposure range. In contrast, the meta-regressions offered little support for concentration-dependent responses across species and exposure scenarios. In practice, high background noise may obscure the small effects even at high exposures, and this problem can only be expected to increase upon extrapolation between different species and exposure scenarios.

Our results will be discussed in terms of sensitivity and robustness to critically address the idea of universal application of these transcripts as biomarkers of general toxicity.

#### **2.17.P-Tu134 Proposition of Reference Range for Genotoxicity Biomarkers in Stickleback by Considering Season and Biotic Factors**

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Biomarkers may reflect a causal relationship between the exposure to chemicals and early impact on organisms. To be employed in freshwater biomonitoring programs at a large scale, natural variability ranges of biomarkers need to be considered. Indeed, the characterisation of reference values faces to limits, such as the influence of confounding factors and the difficulty to find a reference station associated to no or low anthropogenic pressures. Among biological parameters used, the assessment of environmental genotoxicity represents a major challenge due to the wide range of substances which are known to have genotoxic properties on aquatic species. However, the lack of knowledge about the impact of confounding factors on genotoxicity responses can complexify the assessment of freshwater genotoxic risk. The aim of the study was to establish reference baseline values considering potential biotic (sex and length of fish) and abiotic (season) factors. DNA strand breaks, oxidative lesions to DNA (Fpg-sensitive site), DNA content variation, and erythrocyte mortality and density were assessed over a one-year period on the three-spined stickleback, *Gasterosteus aculeatus*, from an outside artificial pond, using the comet assay and flow cytometry. Both Fpg-sensitive site, DNA content and erythrocyte mortality presented sex-dependent seasonal variation. Only Fpg-sensitive site and erythrocyte necrosis were impacted by the fish size. DNA strand breaks and erythrocyte density were not affected by these factors. This work has improved knowledge of erythrocyte biomarkers variability and proposed the first projection of reference range carried out by a mathematical model previously developed. The variability explained by these models, as well as its predictive capacity, vary according to the biomarker considered. The prediction was well represented by the three confounding factors for biomarkers of erythrocyte mortality and chromosomal damages but could be improved by considering other abiotic parameters. A field exposure outside of stickleback breeding periods, with a homogeneous sample (sex ratio and size) should be preconized to improve the genotoxicity assessment. Overall, these results represent the first attempt to define reference ranges for biomarkers of genotoxicity in the stickleback, which will be useful to compare future data from studies of freshwater environments under biomonitoring scopes.

#### **2.17.P-Tu135 Variability of Metallothionein Expression in Field-Populations of *Gammarus fossarum* Living in Long-Term Chronic Cadmium Contamination Contexts: Basal Levels, Sensitivity, and Transgenerational Effects**

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Metallothioneins (MTs) are proposed as biomarkers of metal exposure because MT-synthesis is often induced when organisms are exposed to environments contaminated by heavy metals. Nevertheless, in natural populations, the basal sensitivity of biomarkers is not currently fully characterised whereas it can be variable due to adaptation phenomena and/or influence of past exposure of organisms (acclimatation) or transgenerational effects (parental exposure). This source of variability can make problematic risk assessment in biomonitoring when correlations between the dynamics of metallic exposure and MTs expression in collected organisms have to be drawn. In this way, this study in the sentinel freshwater species *Gammarus fossarum* wants to better understand the plasticity of MT-expression in populations exposed to historical metallic contamination, especially in naturally contaminated river due to geochemical sources. A data mining of recent transcriptomic resources in *G. fossarum* followed by experimental validation by RT-qPCR allowed us to identify two MTs, *GfossMT1* and *GfossMT2*, the first one which responds strongly to Cd exposure in laboratory conditions. Our objectives were to investigate the variability of MT-expression in 13 *G. fossarum* field populations, notably living in long-term chronic Cd contamination contexts, by 1) determining the co-variation between *GfossMT1* expression levels of different life stages and Cd bioavailability in the field, 2) examining whether basal levels of *GfossMT1* expression in organisms maintained in laboratory are dependent on the exposure history of population origin, and 3) characterising under common Cd exposure in controlled conditions the sensitivity of *GfossMT1* expression of

populations with different metallic exposure histories. Our results confirm that *GfossMT1* is overexpressed in naturally populations exposed to historical Cd contamination and suggest that long-term exposure over several generations affects the sensitivity of *GfossMT1* expression to Cd exposure. Our study evidences in the case of *GfossMT1* expression can be the object of adaptive transgenerational processes occurring during historical exposure in field populations.

## 2.17.PC Toward a Better Understanding of Chemical Biomarker Responses in Aquatic Organisms

### Track 3 Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modeling

#### 3.01 Advances in Bioaccumulation Science and Assessment

##### 3.01.T-01 A Tiered Approach for Screening Chemicals for Biomagnification Potential in Air-Breathing Organisms

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Regulatory programs seek to protect human and environmental health and, as a part of hazard assessment, often categorize chemicals according to their persistence (P), bioaccumulation (B), and toxicity (T) properties. For air-breathing organisms, biomagnification is related to the octanol-air partition ratio ( $K_{OA}$ ) in addition to the octanol-water partition ratio ( $K_{OW}$ ). In current REACH guidance, chemicals with  $\log K_{OA} \geq 5$  and  $\log K_{OW} \geq 2$  are considered to have bioaccumulation potential in air-breathing organisms. Critical caveats to these screening criteria are the assumptions that (i) the chemical is efficiently absorbed from the diet, (ii) it is nondissociating (i.e., a neutral organic chemical), and (iii) there is no biotransformation. A better metric to assess bioaccumulation in air-breathing organisms is the biomagnification factor (BMF), defined as the fugacity ratio (applicable to nonionic compound) or the activity ratio of the organism to its diet. As recently outlined by the ECHA working group on toxicokinetics in the “Bioaccumulation assessment of air-breathing mammals: a discussion paper”, physiologically-based biokinetic (PBK) models, describing processes such as chemical absorption, distribution, metabolism (biotransformation), and excretion (ADME), can be used to simulate whole body concentrations at steady-state and calculate the BMF. Here we present a tiered approach for screening organic chemical bioaccumulation in air-breathing organisms. The tiered approach progresses from conservative screening-level assumptions based on  $K_{OW}$  and  $K_{OA}$  to more realistic conditions for chemical properties, internal distribution and biotransformation.

The screening results show a progressive decrease in the chemical activity based biomagnification factor ( $BMF_a$ ) from Tier 0 ( $K_{OA}$  and  $K_{OW}$  only) where 84% of the chemicals predicted as potentially B to Tier 3 when ED, bio-partitioning and dissociation are included in the simulation and 77% of the chemicals have an estimated  $BMF_a > 1$ . Tier 4 shows significantly different results when biotransformation is included in the simulations; biotransformation is by far the main elimination pathway for most chemicals reducing the number of chemicals with  $BMF_a > 1$  to ~2% of the original dataset.

This study shows the key role of biotransformation in bioaccumulation assessment for air-breathing organisms and highlights the need for reliable biotransformation data to effectively categorize chemicals for B hazard.

##### 3.01.T-02 High Throughput Prediction of Hepatic Clearance Using Isolated Perfused Fish Livers in Diverse Chemical Mixtures

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Chemical risk assessment often focuses on screening substances for criteria of persistence, bioaccumulation potential, and toxicity (PBT). Of these criteria, bioaccumulation potential presents unique challenges in effective risk assessment due to the difficulty associated with testing compounds that undergo biotransformation. This difficulty is compounded by the diversity of chemical classes of interest, such as pharmaceuticals, ionizable organic compounds (IOCs), or complex mixtures within the environment. Presently, bioaccumulation assessment relies upon costly whole animal *in vivo* exposures, or new approach methodologies (NAMs) such as *in vitro* assays coupled with *in vivo-in vitro* extrapolation (IVIVE). These approaches have been used to reduce both animal use and uncertainty within assessment frameworks such as REACH, however further refinement is needed to establish the high-throughput predictive tools that are desired by modern chemical legislation. One such refinement involves the use of the isolated perfused rainbow trout liver model at an intermediate level of biological organization. This model can deliver a physiologically relevant measure of hepatic clearance and thus help build an estimate of bioaccumulation potential. In the present study, the hepatic clearance of a diverse mixture of chemicals found within the US EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) trial, as well as nine psychotropic drugs were measured in both isolated perfused trout livers and isolated primary hepatocytes (RT-HEP). Samples were analyzed using liquid-chromatography-high-resolution-mass-spectrometry (LC-HRMS) to semiquantitatively measure individual chemicals within mixture and calculate hepatic extraction fraction. Our results show that the combination of the isolated perfused liver model with mixtures is a powerful predictive tool for investigating bioaccumulation potential across a vast range of chemicals. Coupled with IVIVE modeling, this approach can achieve high-throughput screening of contaminants with minimized animal use and greater certainty than previous studies.

### 3.01.T-03 Elimination Resistant: Characterising Multicompartment Toxicokinetics of Thiacloprid in *Gammarus pulex* Using Bioconcentration and Receptor Binding Assays

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Neonicotinoid insecticides act as agonists of the nicotinic acetylcholine receptors (nAChRs) and induce symptoms of neurotoxicity. In aquatic invertebrates, delayed toxicity is a common phenomenon for neonicotinoids. Further, recent studies observed slow and incomplete elimination of neonicotinoids in amphipods suggesting multicompartment kinetics. However, a mechanistic link between receptor binding and toxicokinetic modelling has not been demonstrated yet.

The incomplete elimination of thiacloprid in the freshwater amphipod *Gammarus pulex* was studied in several toxicokinetic approaches, comprising an extended elimination phase, concentration dependence and pulsed exposure experiments. Further, experiments were complemented with studies on the binding of thiacloprid to nAChRs using *in vitro* and *in vivo* receptor binding assays. Based on the results of the experiments, a toxicokinetic-receptor model was developed to predict the uptake and elimination kinetics of thiacloprid in *G. pulex*.

The performed experiments confirmed the incomplete elimination of thiacloprid in *G. pulex*. The elimination resistant fraction (0.18 to 0.39  $\mu\text{mol/kg}$  wet weight) was independent of exposure concentration and the number of exposure pulses. Based on the receptor binding assays, irreversible binding to the nAChRs was demonstrated to be the major cause of elimination resistance. Accordingly, the toxicokinetic-receptor model consisted of a membrane protein (i.e., nAChR) compartment and a structure compartment. Internal thiacloprid concentration data of various experiments were used to calibrate and validate this toxicokinetic-receptor model.

The present study elucidated the toxicokinetic mechanisms of thiacloprid accumulation in *G. pulex*. Additionally, we provided the tools to assess toxicokinetics including receptor binding. Our results could help in understanding the delayed toxic effects caused by neonicotinoids. Furthermore, we want to raise awareness towards the long-term toxic effects following the irreversible binding to nAChRs where regulatory solutions are needed.

### 3.01.T-04 Using Multimodal Passive Samplers for the Estimation of Risk and Bioaccumulation in *Gammarus pulex*

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A six-month monitoring study for contaminants of emerging concern in an urban river, London UK (River Wandle) using a miniaturised 3D printed passive sampling devices (3D-PSDs) is presented. The 3D-PSDs were manufactured from a methacrylate-based resin and held up to five individual 9 mm sorbent disks. The sampling rates ( $R_s$ ) of three different sorbents (HLB (divinylbenzene-co-N-vinylpyrrolidone), anion exchange, and cation exchange) were assessed in the laboratory over 10 days at low exposure concentrations (50 and 100  $\text{ng L}^{-1}$ ) in artificial freshwater (AFW). The sampling rates ranged from 0.3 to 12.3  $\text{mL day}^{-1}$ , 0.3 to 18.8  $\text{mL day}^{-1}$ , and 2.0 to 81.0  $\text{mL day}^{-1}$  for HLB, anion and cation sorbents, respectively. The 3D-PSDs ( $n = 15$  individual sorbent disks across three devices per sorbent) were deployed for seven days per month from July to December 2021. Appropriate field blanks were used during deployment and retrieval, and water samples were collected at three timepoints over the 3D-PSD deployment. The benthic invertebrate *Gammarus pulex* was collected every month, co-occurring with 3D-PSD deployment and retrieval. All matrices were analysed using a highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Across all matrices, 94 unique compounds were detected, seven of which are watch list compounds. Of these, 14 compounds are common to all matrices, 38 were common to all sorbents, and 19 compounds were common to both the 3D-PSDs and *G. pulex*. Concentrations on the sorbent disks ranged from 0.004  $\text{ng disk}^{-1}$  (mephedrone) to 6.7  $\text{ng disk}^{-1}$  (imidacloprid), 0.02  $\text{ng disk}^{-1}$  (roxithromycin) to 5.0  $\text{ng disk}^{-1}$  (acetamiprid), and 0.02  $\text{ng disk}^{-1}$  (thiacloprid) to 6.3  $\text{ng disk}^{-1}$  (imidacloprid) for HLB, anion and cation sorbents, respectively. The inclusion of the anion and cation phases increased the chemical sampling space by 23 compounds. In the *G. pulex*, concentrations ranged from 3.8  $\text{ng g}^{-1}$  (lidocaine) to 127  $\text{ng g}^{-1}$  (citalopram), median = 10.2  $\text{ng g}^{-1}$ . Risk quotients (RQs) and toxic units (TU) were calculated for all compounds. Imidacloprid was found to have medium to high RQs derived from water and 3D-PSD data and high TU calculated in *G. pulex*. High correlations were found between the effect and toxic units calculated using the *G. pulex* data and mass on 3D-PSD. Multivariate analysis was applied to the *G. pulex* and 3D-PSD data to determine relationships with a view to move toward the prediction of *G. pulex* concentrations from the 3D-PSD data.

### 3.01.T-05 Strengthened Role of New Approach Methods (NAMs) in Bioaccumulation Assessment under REACH

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In recent years, a number of New Approach Methods (NAMs) were developed as alternatives to the bioaccumulation fish tests according to OECD TG 305. The revised ECHA PBT guidance document clarifies their role in the bioaccumulation assessment

and testing strategy. Bioconcentration factors (BCFs) with the aquatic invertebrate *Hyalella azteca* according to the new OECD test guideline, which is scheduled to become available in 2023, can be directly compared against the REACH Annex XIII criteria on bioaccumulation to conclude on bioaccumulative ('B') and very bioaccumulative ('vB'). The results can furthermore be used to conclude on 'not B' or 'not vB', unless there are reasonable doubts that a fish BCF would have exceeded the 'B' or 'vB' thresholds, respectively. In this case, further supporting information is needed. Regarding the *in vitro* methods with fish hepatocytes and liver S9 (OECD TG 319A,B), there is still a lack of practical experience on their performance, and results should be used together with other information to conclude on bioaccumulation. For studying the biotransformation potential of substances in air-breathers, assays with material from mammalian species, such as primary hepatocytes and liver S9, could be used. An ECHA initiated working group on bioaccumulation assessment based on air-breathing species proposed such *in vitro* methods as intermediate tier in a tiered approach to assess the bioaccumulation potential in air-breathing species, with the aim to avoid vertebrate tests as far as possible. Also in this case more experience on its performance is needed, and results should be used together with other information to conclude on bioaccumulation. Assays to determine the rate of hepatic clearance are also used for the assessment of human health systemic toxicity to convert a nominal concentration measured *in vitro* to the equivalent external dose used *in vivo*. Further developments of these assays should aim to combine the needs of both bioaccumulation and hazard assessment for systemic toxicity endpoints. Furthermore, guidance is now available on how to interpret elimination half-lives from toxicokinetic (OECD TG 417) and human biomonitoring studies as indicators of biomagnification. With the update of the ECHA PBT guidance document in 2023, the role of NAMs in bioaccumulation assessment is clarified and advice given on their application in a regulatory context.

### 3.01.P Advances in Bioaccumulation Science and Assessment

#### 3.01.P-Th124 Intelligent Sampling in Standard *in vivo* Toxicity Studies to Complement Bioaccumulation Assessments in Mammals

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Traditionally, a fish bioconcentration factor has been employed to determine the propensity of chemicals to bioaccumulate in organisms in the environment. The requirement to perform a separate assessment of bioaccumulation in terrestrial air-breathing species was introduced in the 2017 revision of the Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11: PBT / vPvB assessment (v3.0) but without technical guidance on how to develop/achieve such an assessment, beyond partitioning-based screening criteria. The ECETOC workshop on Bioaccumulation in Air-Breathing Organisms, held at the SETAC Copenhagen meeting (May, 2022) a tiered assessment approach was proposed and openly discussed, starting from *in silico* screening, progressing to *in vitro* biotransformation assays, then to *in vivo* mammalian studies, as a last option in the event that reasonable doubt remains regarding air-breather bioaccumulation potential. This poster will propose and discuss the merits of smart sampling approaches within standard *in vivo* repeated dose toxicity studies, currently required from Annex VIII and above in REACH. Biological fluids (e.g., plasma, urine), organs, and tissue samples are routinely collected during *in vivo* studies for analytical screening of parent chemicals and their metabolites. These data could be leveraged to develop further weight-of-evidence with respect to body distribution and elimination half-lives of chemicals, both of which are relevant to the bioaccumulation endpoint. The approach respects the "Reduction" principle of the 3Rs, in that more useful data are derived from the same number of test animals since the analytics would be performed on relevant samples obtained from existing standard test protocols (e.g. OECD 407, 408, 421, 422). It is believed that the information derived from this smart sampling approach using amended test guidelines would lead to a significant reduction in the need for separate follow-up targeted bioaccumulation testing in laboratory mammals.

#### 3.01.P-Th125 OECD TG 319B: Comparison of Biotransformation Rates of Organic Chemicals in Liver S9 Subcellular Fractions from Common Carp and Rainbow Trout

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Bioaccumulation in aquatic species in the regulatory assessment of chemicals usually involves the *in vivo* determination of the bioconcentration factor (BCF) in fish (OECD TG 305). Whereas different fish species are recommended *in vivo*, the validated *in vitro* methods using hepatocytes and liver S9 fractions (RT-S9) to determine biotransformation rates (OECD TG 319 A/B) and the *in vitro-in vivo* extrapolation (IVIVE) models have been developed for rainbow trout. For regulatory purposes, different geographical regions may have particular species preferences. As such, complimentary validation work may be required on alternative species to establish OECD 319A/B fit-for-purpose.

The goal of this study was to compare the biotransformation rates of organic chemicals between trout and carp S9 fractions and to compare the predicted BCFs. Substrate depletion assays were performed with 23 different chemicals in carp-S9 (CC-S9) and RT-S9 including pyrene as reference. TG 319 B was slightly modified for the CC-S9: the incubation temperature was 18°C and the pH of the phosphate buffer pH 7.4. Decrease of the test chemicals was analysed by GC-MS.

Four chemicals were not significantly biotransformed in either system and one additional chemical not in RT-S9. For the remaining chemicals, CL<sub>IN VITRO, INT</sub> ranged from 0.04 to 4.01 mL/h/mg protein in CC-S9 (n=19) and from 0.09 to 16.77 mL/h/mg

protein in RT-S9 ( $n = 18$ ). Four of these chemicals were biotransformed slower in CC-S9 by a factor of 3 to 16, while for the majority of chemicals the differences were within a factor of 2. Predicted BCFs using a new empirical regression model which was built with  $CL_{IN\ VITRO,\ INT}$  determined in CC-S9, measured log Kow values and in vivo BCFs determined in common carp, were similar to BCFs which were predicted with a general regression model (i.e., RT-S9  $CL_{IN\ VITRO,\ INT}$ ), log Kow and mixed species BCFs). In addition, a revised IVIVE model from Nichols et al. 2013 was applied to predict BCFs using CC-S9 and RT-S9 data as input with minor adaptations for carp.

The dataset indicates that the differences in  $CL_{IN\ VITRO,\ INT}$  between common carp and rainbow trout are, for the majority of chemicals, within the same range as differences between various batches of RT-S9 and that species matched models do not substantially improve the BCF predictions. In summary, predicted BCF using RT-S9 biotransformation rates may be used to assess bioaccumulation in a “general” fish species.

### 3.01.P-Th126 Is Nanoplastic-Sorbed Tributyltin Bioavailable to Marine Organisms?

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Plastic pollution in our oceans has become one of the most prominent human impacts on the environment over the last few decades. Degradation of plastic debris and the resulting formation of micro- (MP) and nanoplastics (NP) have prompted global interest in modelling the behaviour and environmental impacts of these smaller particles in the environment. Plastic particles are both known to be up taken by marine organisms and have the potential to act as a vector for harmful co-contaminants. But how much of the sorbed contaminant is available to the organism? Can the contaminant reach otherwise inaccessible areas of tissue due to the permeation power of the plastic particle?

One of the co-contaminants of interest is tributyltin (TBT), a legacy aquatic pollutant previously used on the underside of marine vessels as an anti-fouling agent. Toxicity is exhibited to bivalves exposed to concentrations as low as 1 ppb, and the strong affinity to suspended particles in the ocean makes it a perfect candidate to model its variable bioavailability.

In this research, a robust new method for the spatial quantification of TBT in mussel tissue by laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) was developed. Wild blue mussels (*Mytilus edulis*) were exposed to a combination of TBT and 3 various sizes of polystyrene nanoplastic (PS-NP) for 24 h, followed by a 2-week depuration period. Total aqueous and uptaken TBT at several time points were determined by ICP-MS. Given limited availability of matrix-matched reference materials, matrix-matched standards for environmentally realistic concentrations were prepared in house with a combination of spiking, cryogrinding, embedding, and cryosectioning. Whole mussel sagittal sections were mapped via 50  $\mu\text{m}$ -height line scans at a speed of 300  $\mu\text{ms}^{-1}$ . In addition to spatial determination of tin (Sn), maps also were produced for common metal imbalance indicators: copper (Cu), zinc (Zn), manganese (Mn), magnesium (Mg), and iron (Fe).

### 3.01.P-Th127 Can Nanoplastics Carry Harmful Organotins into Aquatic Organisms?

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Plastic pollution in our oceans has become one of the most prominent human impacts on the environment over the last few decades. Degradation of plastic debris and the resulting formation of micro- (MP) and nanoplastics (NP) have prompted global interest in modelling the behaviour of these smaller particles in the environment. MPs have been shown to act as a vector for harmful co-contaminants via chemical and physical adsorption. However, information regarding the adsorption capacity of NPs is somewhat limited, despite their greater penetration power through biological membranes and their larger surface area.

Tributyltin (TBT) is a legacy aquatic pollutant, previously used on the underside of marine vessels as an antifouling agent. Toxicity is exhibited to bivalves exposed to concentrations as low as 1 ppb. TBT has a strong affinity to suspended marine particles such as sediments and plastics. Whilst bound, TBT has an extended half-life of 40 years and therefore exists most in benthic sediments. However, plastic particles are significantly less dense than sediment and may allow for the remobilisation of TBT.

In this research, 3 size classes of Nanopolystyrene (PS-NP) spheres were produced with diameters spanning the nano- range ( $40.09 \pm 0.14$ ,  $483.75 \pm 1.79$  and  $765.52 \pm 9.18$  nm). These PS-NPs were used in combination with ICP-MS and MP-AES during adsorption experiments to explore the kinetics and thermodynamics of TBT adsorption by PS-NP within 4 water matrices including natural sea water (NSW, 32 psu), brackish water (IW, 16 psu), artificial sea water (ASW, 32 psu), and a pH 8 buffer in Milli-Q water. The impacts of other environmental factors such as salinity and PS particle size were also investigated. The majority of TBT adsorption occurred within the first 3-6 hours of mixing. Langmuir and Freundlich isotherm models were plotted for each water and polystyrene particle matrix, with distribution coefficients ( $K_D$ ) ranging between  $192.1 \pm 8.8\ \text{Lg}^{-1}$  and  $2852.8 \pm 290.9\ \text{Lg}^{-1}$ . It was found that the Freundlich isotherm model provided the most accurate fit to experimental data. The greatest adsorption was observed with the smallest plastic particles and in brackish water. This suggests that nanoplastics have a greater potential to act as a vector for the transportation of TBT over microplastics, and that adsorption is restricted by the presence of competitive salts.

### 3.01.P-Th128 Assessing Bioaccumulation Potential of Pharmaceuticals Using Rainbow Trout Liver S9 Intrinsic Clearance

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The OECD 305 is a widely used, standard test guideline for measuring bioaccumulation of chemicals in fish. This test requires a significant number of fish, is expensive, and time-consuming. Therefore, researchers from various government, industry, and academic institutions have dedicated a significant amount of time and resources over the last decade to develop standard methods and models based on *in silico* and *in vitro* approaches to predicting bioaccumulation in fish. *In vitro* systems derived from fish liver tissue were used to predict biotransformation, a key factor in understanding the bioaccumulation of chemicals in fish. A major scientific accomplishment in this area of research was the development and validation of OECD 319B, "Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 subcellular fraction (RT-S9)", adopted June 2018.

Neutral organic chemicals were used to develop and evaluate the OECD 319B Guideline; therefore, additional research is required to evaluate and optimize the extrapolation model for use with ionic organic chemicals (IOCs), such as pharmaceuticals. The procedures as outlined in the guideline were used to determine *in vitro* intrinsic clearance rates for several pharmaceuticals. These rates were then used to estimate fish BCF values for comparison with the *in vivo* fish BCFs determined as per the OECD 305 Guideline. The predicted BCF values derived from the *in vitro* approach are presented along with the *in vivo* BCF data.

### 3.01.P-Th129 Evaluation of the Ema Trigger for Fish BCF Testing: Evaluation of Log D and Fish Bcf Data for Several Pharmaceuticals

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As per the European Medicines Agency (EMA) Guideline for Environmental Risk Assessment of Medicinal Products for Human Use, a fish bioconcentration study is triggered in Phase I for pharmaceuticals having a log Kow >4.5 to support screening for Persistence, Bioaccumulation, and Toxicity (PBT) and in Phase II for pharmaceuticals having a log Kow >3 to assess bioaccumulation potential. The recommended protocol for bioaccumulation is OECD Test Guideline 305: Bioaccumulation in Fish, Aqueous and Dietary Exposure. Based on the standard OECD 305 sampling schedule, approximately 200 fish per study may be required to determine steady-state (BCFSS) and kinetic (BCFK) bioconcentration factors following fish exposures to a low- and high-test concentration and a negative control. As per the draft revision of the EMA guideline, released for consultation in 2018, when log Kow is  $\geq 3$  the potential for secondary poisoning should be evaluated by first determining the experimental fish BCF, and when the resulting BCF is  $>100$  L kg<sup>-1</sup>, a secondary poisoning assessment is required. Considering the potential for fish to metabolize and excrete xenobiotics and the number of animals required for a standard BCF test, the BCF values for several pharmaceuticals were evaluated to understand whether existing data support the current log Kow trigger of 3 for BCF testing. In the interest of animal welfare, BCF data were also evaluated to determine if study design could be limited to a single test concentration and still provide a good estimate of the BCF while reducing the number of fish required. Based on the data presented, increasing the log Kow trigger for BCF testing from 3 to 4 is proposed, and use of a single test concentration to evaluate bioaccumulation potential is recommended.

### 3.01.P-Th130 Toxicokinetics and Biotransformation in *Eisenia fetida*: Comparison of a Short Exposure Design with the Standard OECD Bioaccumulation Study

**Oihane Del Puerto Bengoetxea**, Syngenta Crop Protection, Basel, Switzerland

In bioaccumulation studies for risk assessment, a kinetic bioaccumulation factor ( $BAF_k$ ) is calculated as the ratio between the uptake rate constant and the elimination rate constant assuming first-order kinetics. This implicitly also assumes that it is not needed to reach steady state conditions, and thus the kinetic bioaccumulation does not depend on the duration of the experimental uptake and elimination phases and not on the exposure concentrations. If this holds true, then the kinetic bioaccumulation factor could be estimated based on a shorter experimental time than the one suggested by the OECD guideline. However, we do not know if the key assumption on steady state is generally true and consequently, we do not know how reliable  $BAF_k$  estimates from shorter experiments would be. If the  $BAF_k$  could reliably be estimated from a shorter experiment, it would substantially decrease the costs and time of experimental work. Hence, the aim of the present study is to compare results from a short experimental design and the usual bioaccumulation study design following OECD.

A laboratory study was carried out in which single *Eisenia fetida* earthworms were exposed to three organic compounds with different physicochemical properties (Log P between 2-4). The study consisted of two parallel experiments named "short" and "long". The short experiment consisted of 7 days of exposure in the uptake phase and 14 days in the elimination phase, whereas the exposure duration of the long experiment in the uptake phase was set to 21 days followed by 14 days elimination phase. The compounds were extracted from soil, porewater, and whole earthworms and quantified by liquid chromatography coupled with mass spectrometer. A one-compartment TK model was fitted to estimate uptake and elimination rate constants and  $BAF_k$  were derived. In addition, a suspect screening approach was used to detect possible metabolites and estimate biotransformation rate constants.

Kinetic rate constants and  $BAF_k$  values were obtained from the short and long experiments for the same compound and compared between the experimental designs and across the test compounds. We compare the suspected metabolites and the respective enzymatic pathways of the different test compounds. Finally, we will also discuss the feasibility and consequences of decreasing



the experimental duration for the reliability of estimates of kinetic rate constants and the respective kinetic bioaccumulation factors.

### 3.01.P-Th131 Organ-Specific Biotransformation in Salmonids: Insight into Enzyme Kinetics and Micropollutant Clearance

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For aquatic animals, like fish, bioaccumulation assessments have largely focused on the biotransformation capacity of the liver, as the organ responsible for a large proportion of the biotransformation activity. However, limited information exists regarding extrahepatic biotransformation and its potential influence in estimating bioaccumulation parameters. To explore the ability of different organs to support biotransformation processes, we isolated S9 subcellular fractions from the liver, intestine, gills, and brain of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), and conducted comparative evaluations of biotransformation enzyme kinetics and intrinsic clearance of two micropollutants: the fungicide azoxystrobin and the pharmaceutical propranolol. In addition, pyrene was included as a well-studied reference chemical. Our observations suggest significant phase I and II constitutive enzyme activity in all organs of both species, particularly for CYP1A and Glutathione-S-transferase (GST), respectively. CYP2B and UDP-glucuronosyltransferase (UGT) displayed similar activity magnitude in the liver and the intestine. For rainbow trout in specific, CYP2B displayed the highest activity in the gills. Neither CYP2B nor UGT were detected in the brain. All chemicals tested were biotransformed by liver S9 fractions. However, interspecific differences were observed regarding the ability of different organs to biotransform the compounds. Significant extrahepatic biotransformation was observed for pyrene, mainly in brown trout. However, extrahepatic biotransformation in this species was not significant for the two tested micropollutants. In rainbow trout, both the intestine and the brain appeared to biotransform azoxystrobin. Similarly, propranolol appeared to be biotransformed by the intestine and gills. Altogether, these observations provide evidence that extrahepatic biotransformation may depend on the presence of tissue-specific enzymes and their ability to interact with chemicals. Thus, considering the biotransformation potential of different organs could help in refining the estimation of bioaccumulation parameters during environmental hazard and risk assessments.

### 3.01.P-Th132 A Comparison of *in vitro* Metabolic Clearance of Various Regulatory Fish Species Using Hepatic S9

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*In vitro* metabolic clearance rates are used in a combination approach with QSAR modeling and *in vivo* testing with whole organisms for bioaccumulation predictions. However, estimations from QSAR modeling are predicated from databases and suffer from variability of data from inconsistent testing protocols and variations of tested species. OECD 319B is an *in vitro* assay that only covers rainbow trout hepatic clearance. Despite accuracy in whole organisms, fiscal limitations, temporal limitations, and new mandates from the European Union regarding animal welfare are noteworthy drawbacks for *in vivo* testing. Herein, we elucidate the concordance of rainbow trout hepatic S9 fractions to other regulatory species, considering the potential differences in metabolism between warm- and cold-water species. In addition to analyzing the *in vitro* clearance rates of seven chemicals crossing multiple classes of chemistry and modes of action, the S9 exposure methods were refined, generating a more robust and accurate method. The rainbow trout *in vitro* S9 clearance rates of 4-nonylphenol, amitraz, chlorpyrifos, fenthion, pyrene, and S-indoxacarb are equal or superior to that of other tested species. These results indicate that rainbow trout S9 fractions have the potential to be used as surrogates for multiple piscine species of regulatory concern when predicting BCFs. Further, we show that Carp and Fathead Minnow showed similar metabolism to Rainbow Trout and can be used as warm water species for predicting BCF. This research can be leveraged prior to or in place of initiating a TG305 BCF study to aid in selection of the appropriate regulatory model.

### 3.01.P-Th133 Use of Primary Rainbow Trout Hepatocytes to Determine *in vitro* Intrinsic Clearance for Bioaccumulation Assessment in Fish

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In order to improve *in silico* predictions of chemical bioaccumulation in fish, methods are needed to estimate hepatic biotransformation and incorporate this information into established computational models. One promising approach involves the measurement of intrinsic clearance using *in vitro* biotransformation systems derived from liver tissue. The OECD Test Guidelines (TG) 319A and B support this approach and therefore contribute to alternative methods to animal experiments in context of the 3Rs.

Primary Rainbow trout hepatocytes (*Oncorhynchus mykiss*) were isolated from animals kept under controlled environmental conditions and cryopreserved. Cells remained a high viability after thawing over a time frame of at least 2-3 years and kept viabilities above 90 % under shaking conditions (1000 rpm, 14 °C) for metabolism and depletion assays in suspension cultures. The metabolites of three main Cytochrome P450 reactions as well as glucuronidation and sulfation reactions were quantified.

Furthermore, the *in vitro* intrinsic clearance was determined with the reference chemical Pyrene (0.025  $\mu\text{M}$ ) using the cells in substrate depletion assays according to the OECD TG

319 A with direct comparison to heat-inactivated hepatocytes as negative control. Incubations were performed with  $2 \times 10^6$  viable cells/mL for up to 15 minutes at 11 °C. Pyrene concentration was analyzed by GC-MS with anthracene as internal standard for quantification. The measured *in vitro* intrinsic clearance values ( $\text{CL}_{\text{IN VITRO,int}}$  of 2.78-5.41  $\text{mL} \cdot \text{h}^{-1} \cdot 10^6 \text{ cells}^{-1}$ ) were comparable to results from the international ring trial. These  $\text{CL}_{\text{IN VITRO,int}}$  can be used to extrapolate to the whole body biotransformation rate to estimate a BCF (bioconcentration factor) using *in vitro* to *in vivo* extrapolation models or as direct inputs in physiologically based toxicokinetic models (PBTK) for fish bioaccumulation assessment.

Primary hepatocytes from Rainbow trout and other fish species, e.g., Atlantic salmon or Common carp, as well as from other species like chicken or duck, are becoming increasingly important *in vitro* models as alternatives to animal testing in the field of environmental toxicology, in particular, for bioaccumulation assessment.

### 3.01.P-Th134 Kinetics of Uptake and Elimination of Complex Drug Mixture in Zebrafish (*Danio rerio*)

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Active pharmaceutical ingredients (APIs) belong to the most frequently detected categories of emerging environmental contaminants in the aquatic environment. However, in contrast to the large number of reports dealing with the occurrence and fate of APIs in abiotic environmental matrices, data on their concentrations in aquatic organisms are still relatively scarce. In this context, it is essential to understand and assess their bioconcentration potential as well as the uptake and elimination kinetics of APIs at realistic conditions, including environmentally relevant concentration levels and simultaneous exposure to complex mixtures of APIs.

In this work, bioconcentration and elimination of 20 different APIs, covering a wide range of therapeutic categories and physicochemical properties, were studied under controlled laboratory conditions using zebrafish (*Danio rerio*) as a model organism. The exposure was performed at a concentration of 10  $\mu\text{g/L}$  of each API in the mixture and involved a 7-day uptake period followed by a 7-day purification period. Samples of fish and water were collected daily and analyzed using a thoroughly validated multiresidue LC/MS methodology for both matrices, which allowed sensitive and accurate determination of APIs and their biotransformation products. To verify the preset exposure concentrations, water samples were processed immediately, while whole fish samples were frozen using liquid nitrogen, cryogrinded, and stored at -80 °C until analysis. The results showed that the kinetics of API uptake was relatively fast, reaching a plateau for most of the APIs within 7 days. However, the bioconcentration factors of selected target APIs varied in a rather wide range. The most pronounced bioconcentration was observed for sertraline, oxazepam, and desloratadine with bioconcentration factors (BCF) reaching 55, 22, and 15, respectively. The bioconcentration of APIs was associated with a slight to moderate increase in CYP1A and CYP3A activity in API-exposed fish. The elimination of APIs, after 7 days of exposure was also relatively fast and reached rather low levels ( $\leq 5\%$  of the maximum) within the first 2-3 days. The exception was azithromycin, caffeine, and desloratadine whose residual concentrations on the 7th day were around 27%, 18%, and 7%, respectively.

### 3.01.P-Th135 Intrinsic Labelling to Track and Quantify Bioaccumulation and Tissue Distribution of Nanoplastic in Biological System: Case Study of Rainbow Trout

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Radiolabelled NP have been successfully used to overcome the significant limitations for quantifying carbon-based contaminants in environmental and biological media and to facilitate work at low limits of detection. For instance, nuclear techniques such as the quantitative whole-body autoradiography (QWBA) offer the possibility for direct visualization of intra-organ radiolabelled-particle concentrations. In the study presented here, we radiolabelled the carbon-backbone of the NP by synthesizing  $^{14}\text{C}$ -labeled nanopolystyrene particles (*n*PS, 20 and 250 nm), and then exposed Rainbow trout to these particles, measured the biokinetics and quantified NP tissue distributions via QWBA. The aim of the study was to compare how toxicokinetic and tissue distribution of nanopolystyrene would differ at different dosing and times of exposure. We used environmentally realistic *n*PS concentrations (15 ppb) and concentrations 17 times higher (250 ppb) for low and high dosing, respectively. This allowed us to test the hypothesis that Rainbow trout, a commercially important fish, might uptake, absorb, and depurate NP differently at different dosing and exposure times. Our experiments showed that, even at a concentration of 15  $\mu\text{g L}^{-1}$ , *n*PS accumulated rapidly within the lumen of the digestive tract in fish, but that accumulation in internal tissues including skeletal muscle (i.e., fillets) was minimal. Tissue and faeces toxicokinetics show differences between acute and short-chronic exposures and suggest differences in accumulation and depuration. Once the fish transferred to clean food pellets only, the radioactive particles were depurated relatively rapidly from the fish. The potential for both sizes of *n*PS to transfer in the circulatory system is suggested by their presence (although at minimal levels of detection), revealed on the QWBA, in bones, eyes, skin, kidney. This emphasizes the advantages of using radiolabelling techniques in the study of Contaminants of Emerging Concerns (CEC). The use of radiotracers will help to further the understanding of bioavailability, bioconcentration, bioaccumulation, biotransformation, and biomagnification of organic CEC (e.g., NP, p-phenylenediamine (PPD) additives, Perfluoroalkyl and Polyfluoroalkyl Substances (PFAs), Tributyl tin) in biota. The type of data that this approach produces is critical to inform risk assessments to develop policies surrounding those chemicals.

### 3.01.P-Th136 Cross-Species Evaluation of Bioaccumulation Thresholds for Air-Breathing Animals

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In air-breathing organisms, an organic chemical's susceptibility for elimination via urinary excretion and respiratory exhalation can be judged based on the octanol–water partition ratio ( $K_{OW}$ ) and the octanol–air partition ratio  $K_{OA}$ , respectively. Current regulations specify that chemicals with  $K_{OW} < 10^2$  and  $K_{OA} < 10^5$  may be screened as nonbioaccumulative in air-breathers. These thresholds are based on model simulations of bioaccumulation in food webs with primarily large warm-blooded, carnivorous mammals as top predators. Their ability to eliminate chemicals may differ greatly from animals with different feeding habits, thermal physiology, or metabolic activity. It therefore remains possible that current  $K_{OW}$  and  $K_{OA}$  thresholds are not protective of all air-breathing species. Here we used a model-based approach to derive threshold values consistent with a biomagnification factor equal to 1 for 140 different mammals, birds, and reptiles. Animals with lower rates of respiration (e.g., manatees, sloths) and those ingesting a high lipid diet (e.g., polar bears, carnivorous birds) were predicted to be able to biomagnify chemicals with  $K_{OA}$  below  $10^5$ . This was also the case for several cold-temperature acclimated reptiles due to lower reported respiration rates and internal temperatures. Protective  $K_{OA}$  thresholds for each animal group were determined to be  $< 10^{4.80}$  (mammals),  $< 10^{4.70}$  (birds),  $< 10^{4.75}$  (reptiles at  $> 25^\circ\text{C}$ ), and  $< 10^{3.35}$  (reptiles at  $\leq 25^\circ\text{C}$ ). For all animals, urination alone was not efficient to prevent biomagnification of any organic chemical as we found no  $K_{OW}$  below which a chemical cannot bioaccumulate in air-breathers. For chemicals with a  $K_{OW}$  below  $10^1$ , we found that biomagnification of persistent chemicals was constrained by the water–air partition ratio ( $K_{WA}$ ) rather than the  $K_{OA}$ . Differences in physiology may need to be considered when assessing bioaccumulation in air-breathing species.

### 3.01.P-Th137 *In vitro* Approach to Refine Bioconcentration and Biotransformation Predictions of Organic Persistent Pollutants Using Cell Lines

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Understanding the processes of bioaccumulation and biotransformation in an organism, and the relationship between them is crucial for assessing the toxicity and therefore the regulation of chemical compounds. In recent years, there has been a strong interest in developing efficient and cost-effective tools that use alternative *in vivo*, *in vitro*, and *in silico* assays to predict human health effects, reducing animal testing. The tests with primary hepatocyte (RT-HEP) and S9 fractions (RT-S9) of rainbow trout, with a substrate depletion approach, has been validated (OECD TG 319 A and 319B) to estimate the biotransformation rate and refine BCF predictions using *in vitro-in vivo* extrapolation (IVIVE). However, these extrapolations and assumptions (e.g., the free concentration is fully bioavailable to the cell and only for the metabolization process and not considering the bioaccumulation in *in vitro* systems) continues to over- or underestimate BCF values comparing with those obtained by official OCDE 305 bioaccumulation test guide (<https://doi.org/10.1002/etc.4791>). Therefore, to improve *in silico* predictions, further experimental data is needed to understand all the mechanisms involved in these processes.

The studies presented here are based on the determination of the substrate concentration in the medium and inside the cells, as well as that of its major metabolites to assess the bioavailable concentration and the actual biotransformation rate. These determinations are carried out at different times of the assay in aim to model the behaviour and kinetics of the substrate and its metabolites depletion and formation, respectively. Liver cell lines such as HepG2 (human hepatoma) or ZFL (zebrafish liver) are used in these assays because they allow assessment of cell bioavailable concentration and biotransformation; evaluation in animals other than fish; longer incubations (up to 72 h) that allow evaluation of compounds with slower metabolism rates; as well as their continuous use without the need for animal experimentation.

The results shown focused on PAHs and PBDEs, known persistent organic pollutants (POPs), as well as that of their mayor metabolites (OH-PAHs, OH-BDEs, and MeO-BDEs), contribute to the improvement of IVIVE models to reduce animal suffering trials and to a better understanding of the metabolic pathways and toxicity of POPs.

### 3.01.P-Th138 Relationship Between the BMF and BCF

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The Bioconcentration Factor (BCF) has played a key role in identifying the bioaccumulation potential of organic substances in aquatic organisms. However, the BCF has some key limitations, including its onerous and difficult measurement in laboratory tests (especially for very hydrophobic substances); limited domain of applicability (i.e., water breathing organisms only); and lack of thermodynamically or biologically relevant criteria values that can be used to determine if a substance is truly bioaccumulative. These limitations have become more apparent with the increasing variety of chemicals that are being assessed today and cause both over and underestimations of the bioaccumulation potential of chemical substances that are best avoided. It has been proposed that the biomagnification factor (BMF) is a more generally applicable metric (i.e. applies to water and air-breathing organisms) with thermodynamically and biologically relevant criteria values, that can be measured in laboratory based tests that are simpler than BCF tests and can also be derived from field data. One of the key questions for using the BMF in bioaccumulation assessments is how the BMF compares to the BCF and what this means for bioaccumulation assessment. To address this question, we compiled data from 252 dietary and 350 aqueous bioaccumulation studies in fish for a wide range of substances in fish to (i) investigate the relationship between the BCF and the BMF for the same substance in the same fish species; and (ii) to investigate methods for deriving the BCF from the results of dietary bioaccumulation tests. The results of this

analysis show that BCFs in fish can be derived from BMFs in fish with good precision, but that simple empirical correlations between the BCF and BMF are of limited use for bioaccumulation assessment.

### **3.01.P-Th139 A Food Web Bioaccumulation Model for Quantifying the Dietary Exposure to Persistent Organic Pollutants of Beluga Whales from the St. Lawrence Estuary, Quebec, Canada**

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The elevated levels of persistent organic pollutants (POPs) in beluga whales (*Delphinapterus leucas*) of the St. Lawrence Estuary (SLE), classified as an endangered species, may be linked to their current population stagnation, despite many recovery efforts. However, a quantitative assessment of the bioaccumulation of POPs in the aquatic food web leading up to the SLE beluga whales has not yet been done. Therefore, further understanding the diet of the SLE belugas and modeling the bioaccumulation of contaminants through their food web may help shed light on the pathways of dietary exposure of these whales. Here, we construct a food web with feeding relationships and diet compositions of male and female SLE beluga whales using previously reported stomach contents data and staple isotopic ratio measurements (i.e.,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) in beluga muscle tissue. Eleven potential prey species of the belugas were identified, whose feeding relationships, diet composition, and trophic position were estimated from the diets reported for each species in the literature. Using these feeding preference matrices of the aquatic food web and organism-specific characteristics collected from the literature, we parameterized a previously published food web model, which utilises a mass balance approach to calculate steady-state concentrations of lipophilic chemicals in each of the organisms. The final assembled food web model includes phytoplankton, zooplankton (copepods and krill), seven invertebrate species, nine fish species, and different age classes and sexes of beluga whales (adult and juvenile male, adult and juvenile female, infants). We applied the model to quantify the bioaccumulation of polychlorinated biphenyls and perfluoroalkyl sulfonamides in the food web of the SLE belugas. We also compare the predicted results with measured concentrations of these compounds in the whales and other aquatic species of the SLE to evaluate model performance.

### **3.01.P-Th140 Using *in silico* Bioaccumulation Models: Review and Comparison of the EPI Suite BCFBAF and EAS-E Suite BET**

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Bioaccumulation (B) assessments using various methods, data, metrics, and classification criteria are routinely conducted as a part of chemical regulatory programs. Various screening-level tools are available that incorporate measured and estimated B metrics. The BCFBAF module in EPI Suite v4.11 has two models, BCFWIN fragment-based approach and the 2003 Arnot & Gobas bioconcentration (BCF) and field bioaccumulation factors (BAFs) model. The Bioaccumulation Estimation Tool (BET) is a screening-level mechanistic modelling system integrated in the Exposure And Safety Estimation (EAS-E) Suite platform. The BET is based on mass balance bioaccumulation models in the BAT ver.2.0 including representative food webs (aquatic and terrestrial) and ecological receptors (plants, invertebrates, fish, birds, and mammals). Typical lab test animals are also included, i.e., fish and rat. B-metrics including BCF, BAF and Biomagnification Factor (BMF) for both neutral and ionogenic chemicals, such as PFAS can be predicted for all organisms.

A bioaccumulation database of field and laboratory B metrics has been collected and reviewed. Over 400 organic neutral and ionogenic chemicals spanning partitioning space and biotransformation half-lives have been included. The physical chemical properties were obtained from within each modelling system.

The models give similar preliminary predictions of logBCF, though using differing approaches. Expanding the database to include all the BCFBAF training set chemicals will give further insight into the predictive ability of the BET. The BET predicts slightly higher logBAFs up to a value of 5 or 6 and lower values above that, though still well above any threshold values. BET captures decreased bioaccumulation of IOCs.

Preliminary analysis shows that Aquatic BMFs are predicted with slight positive bias (0.88) and low RMSE (10.16). We hope to present analysis of terrestrial BMFs predicted by the model as well.

We compared a regression-based model with a mechanistic model and achieved similar results. Extending B-assessments to include BMFs from both aquatic and air-breathing organisms is useful for identifying hazards of a wider variety of chemicals. *In silico* tools such as the EAS-E Suite BET can be utilized to guide these assessments with confidence.

### **3.01.P-Th141 Localising Organic Contaminants and their Biotransformation Products in Whole Body Cross-Sections of Aquatic Invertebrates Using Two Mass Spectrometry Imaging (MSI) Techniques**

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Elucidation of spatial and organ specific distribution of drugs in an organism by mass spectrometry imaging (MSI) is an intensively applied method in medical sciences. A transfer of this method toward environmental chemistry would allow the

localisation and discrimination between parent contaminants and their biotransformation products (BTPs) in nontarget organisms, such as the aquatic amphipod species *Gammarus pulex*. Further, MSI allows the analysis of multiple compounds within the same sample. The present study aimed to assess the spatial distribution of organic contaminants and their BTPs in the tissue *G. pulex* by applying different MSI and dissection methods, complemented by online solid phase extraction coupled with liquid chromatography and high resolution tandem mass spectrometry (online SPE-LC-HRMS/MS).

Specimens of *G. pulex* were exposed in water containing a mixture of pesticides (cyprodinil, fluopyram, terbutryn) and pharmaceuticals (carbamazepine, citalopram, diclofenac). Whole body cryosections (16 µm thickness) were analysed by matrix-assisted laser desorption ionisation (MALDI-) MSI or by desorption electrospray ionization (DESI-) MSI. Additionally, extracts from dissected and nondissected gammarids were analysed online SPE-LC-HRMS/MS to confirm the internal concentrations of different compartments.

The whole body internal concentrations determined by LC-HRMS/MS ranged from 2.5 µg/g (citalopram) to 14 µg/g (cyprodinil). Five of the six compounds (except diclofenac) could be detected using DESI-MSI whereas only three (citalopram, cyprodinil, terbutryn) could be detected using MALDI-MSI. The parent compounds generally followed a rather uniform distribution in the amphipod tissue, indicating a passive diffusion driven bioaccumulation. Several BTPs of terbutryn and cyprodinil were detected in the gastrointestinal system of the cross-sections analysed by MALDI-MSI. The observation was supported by the LC-HRMS/MS analysis of dissected specimens. This revealed the intestine to be the main biotransformation site in gammarids.

The present study demonstrates the suitability of MSI for investigations on the spatial distribution of organic contaminants and biotransformation sites in *G. pulex*. However, both applied methods required relatively high tissue concentrations and DESI-MSI appeared to be the more sensitive method. Further improvements would be needed to address environmental concentrations.

### **3.01.P-Th142 Determination of Engineered Nanomaterial Bioavailability in Amphipods Using Haemolymph Isolation and Single Particle ICP-MS**

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Currently, tiered approaches using invertebrates are discussed to avoid animal tests for regulatory bioaccumulation assessment such as the fish bioaccumulation tests (OECD TG 305). The *Hyalella azteca* bioaccumulation test (HYBIT) has been successfully used in a series of bioaccumulation studies with engineered nanomaterials (ENMs). However, due to the small size of the amphipod, it is not possible to distinguish the ENMs in the intestinal content from the real incorporated, bioavailable fraction (ENM in the tissue or body fluids). Even if a concept for a tiered assessment scheme based on ENM-HYBIT that takes this ambiguity into account with adjusted endpoints exists, further data on the bioavailability of the tested ENMs are of high value. However, imaging techniques for the localization of tissue-incorporated ENMs require sophisticated time- or cost-intensive analytical methods or very high exposure and body burden concentrations (e.g., correlative microscopy or micro X-ray fluorescence imaging).

Within this study, a microcapillary based simple method that can be used in any laboratory was developed isolating the haemolymph of exposed amphipods. *H. azteca* was exposed to AgENMs and AgNO<sub>3</sub> (ionic comparison group). After exposure, the haemolymph from 20 animals per replicate was collected, pooled, diluted, and analyzed for the presence of AgENMs using single particle Inductively Coupled Plasma-Mass Spectrometry (spICP-MS). By this, we were able to measure AgENMs in the haemolymph, despite the small sample volume. The measured AgENMs showed a strong comparability in size to the particles measured in the exposure medium. Ag in particulate form was also found in the haemolymph from the AgNO<sub>3</sub> treatment, but these were a different size and their size distribution was more heterogeneous. This indicates the formation of secondary particles, as has already been described for other species after AgNO<sub>3</sub> exposure.

The results show that the method developed is suitable for assessing the bioavailability and tissue translocation of ENMs. Secondary particles (particles generated by precipitation of Ag in the medium or in the organism) as well as pseudoparticles can be identified by comparison with spICP-MS measurements of the exposure media. This approach provides valuable insight that can support data interpretation from the ENM-HYBIT to provide a higher degree of certainty for the assessments.

### **3.01.P-Th143 Mobilization of POPs in Humpback Whales from Feeding Areas Around the Antarctic Peninsula and Strait of Magellan and Migration, Breeding, and Calving Grounds on the Brazilian Coast**

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The Southern Hemisphere humpback whales (*Megaptera novaeangliae*) face the longest migration distances and fasting periods that reflect the composition of the lipophilic contaminants to which they are exposed. To evaluate these changes, persistent organic pollutants (POPs) were determined in blubber samples of humpback whales around the Antarctic Peninsula (n=46), in the Strait of Magellan, Chile (n=22), and off the Brazilian coast (n=38). The similarity in the PCB and HCB contents in the individuals from feeding (Stock G) and breeding (Stock A) grounds indicates that they may have been contaminated while feeding in higher latitudes. In the whales around the Antarctic Peninsula, the predominance of tetrachlorobiphenyl PCBs with a marked presence of di- and tri-biphenyl congeners was evident. The whales that feed in the Strait of Magellan showed a slight predominance of 5Cl biphenyls as a consequence of their feeding on subantarctic krill species but also on small fishes and galatheid crabs that could be contaminated by industrial activities in Chile. The dominance of 5-6Cl congeners in the whales of Stock A in Brazil and the lack of 3-4Cl congeners is probably related to the extreme physiological changes during fasting when

whales mobilize blubber reserves and metabolize lighter congeners or by transferring them to their calves, even though they can feed opportunistically along migratory routes.

### **3.01.P-Th144 Mercury and Selenium Concentrations, and Selenium:Mercury Molar Ratios in Embryos of Three Placental Viviparous Shark Species (*Carcharhinus leucas*, *Carcharhinus limbatus*, and *Carcharhinus plumbeus*)**

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Embryos from placental viviparous shark species accumulate high concentrations of mercury (Hg) due to maternal transfer; however, the resulting impacts on embryonic development are not known. It has been argued that selenium (Se), an essential trace element, has a protective effect against Hg toxicity if the Se:Hg molar ratio is >1:1; therefore, sharks may be protected against the adverse health effects of Hg exposure during embryonic development if Se is in molar excess of Hg. The goal of this study was to 1) investigate the inter- and intraspecies variability in Hg and Se concentrations, and Se:Hg molar ratios in muscle, heart, brain, liver, and kidney of bull shark (*Carcharhinus leucas*), blacktip shark (*Carcharhinus limbatus*), and sandbar shark (*Carcharhinus plumbeus*) embryos, and 2) compare the Hg and Se concentrations, and Se:Hg molar ratios in muscle and liver of each embryo to the values for their corresponding parent. Mercury concentrations were measured using a Direct Mercury Analyzer and Se concentrations were determined using microwave acid digestion and ICP-MS analysis. Three pregnant bull sharks (n = 16 embryos), four pregnant blacktip sharks (n = 20 embryos), and four pregnant sandbar sharks (n = 37 embryos) were included in this study. For all species, the mean ranking of embryo Hg tissue concentrations was muscle > heart > kidney > brain > liver. Bull shark embryos had the greatest mean muscle, heart, brain, and liver Hg concentrations, whereas blacktip shark embryos had the greatest mean liver Hg concentration. There was no clear tissue ranking in Se concentrations; however, the mean concentration was greatest in the kidney in all species. Among species, mean embryonic muscle and liver Hg concentrations were between 10.6% to 21.8% and 0.33% to 0.68% of the parent's mean muscle and liver concentration, respectively, whereas Se was between 206% to 404% and 46.6% to 114%, respectively. For all species, the mean embryonic Se:Hg molar ratio ranking was liver (167-613:1) > kidney > brain > muscle > heart (28-72:1). Sandbar shark embryos had the greatest mean liver, heart, brain, and kidney Se:Hg molar ratios, and lowest muscle Se:Hg molar ratio. In comparison, mean muscle and liver Se:Hg molar ratios were just above 1:1 in parental tissues of each species. The high Se:Hg molar ratios indicate that Se may have a protective effect against Hg toxicity during embryonic development, reducing the adverse effects of Hg on growth and development.

### **3.01.P-Th145 Integrated Assessment and Testing Strategy for Bioaccumulation Assessment Under REACH: New Pathways to Weight-of-Evidence**

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With the update of the ECHA PBT guidance in 2023, the integrated assessment and testing strategy for bioaccumulation assessment was revised to take account of the technological and scientific progress in recent years. A number of New Approach Methods (NAMs) were developed as alternatives to the fish BCF test (OECD TG 305). In the future, the freshwater amphipod *Hyalella azteca* bioconcentration test (HYBIT) should be considered, where possible, to avoid vertebrate testing. *Hyalella* BCF values can be compared against the REACH Annex XIII criteria for bioaccumulation, i.e., bioaccumulative ('B') and very bioaccumulative ('vB'). The result can furthermore be used to conclude on 'not B' or 'not vB'. If it cannot be excluded that due to the lower lipid content of the amphipods the bioaccumulation potential of a substance may be underestimated compared to fish, further information is needed. For highly hydrophobic substances, the dietary exposure bioaccumulation fish test (OECD TG 305) has been suggested. The ECHA PBT guidance recommends that the elimination rate constants derived from fish feeding studies should be used to estimate aquatic BCF using the methods described in Annex 8 of OECD TG 305 and its related Guidance Document. Furthermore, lipid correction for the biomagnification endpoint is discussed. Growth correction is supported and further explained. It is noted that high molecular weight is no longer accepted as an indicator for hindered uptake. Biomagnification and corresponding elimination half-life are the key endpoints for bioaccumulation assessment of terrestrial and air-breathing organisms. According to the ECHA PBT guidance document, the use of mammalian *in vitro* tests for the estimation of biotransformation rates is a promising pathway to be developed further to support bioaccumulation assessment with air-breathing organisms. Moreover, the PBT guidance document gives advice on the interpretation of elimination half-lives derived from toxicokinetic studies according to OECD TG 417 and human biomonitoring studies. In addition to that, the revised guidance document includes also more detailed guidance on the use of field and biomonitoring data, assessment of ionisable substances and surfactants. The poster gives an overview of the new pathways to weight-of-evidence covered by the updated ECHA PBT guidance document with respect to bioaccumulation assessment.

### **3.01.P-Th146 Integrated Strategy for the Assessment of Aquatic and Terrestrial Bioaccumulation**

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A concept for an intelligent crosscutting assessment strategy for bioaccumulation in the context of the prospective assessment of substance regulations (chemicals, plant protection products, biocides, and pharmaceuticals) incorporating the findings from

previous projects of the UBA, the ECHA, and other institutions is presented. The strategy permits the protection of the environment at an early stage against accumulating substances, especially against accumulation in the food chain and with regard to the risk of secondary poisoning. Hazard-based and risk-based assessment approaches are considered. A more efficient and accurate identification of bioaccumulative substances will permit an improved identification of substances with PBT and/or vPvB properties. In addition, the strategy will help to advance alternative methods to the existing tests with vertebrates (e.g. OECD 305) and to integrate them into the assessment, including the investigation of their uncertainties. Such alternative testing and assessment methods are required and can be useful in several regards: They are meant to minimise the use of animal testing, can enable improved informative value for extrapolation to ecosystems and transferability to species other than those tested. This will result in more reliable estimates based on less data and indicate for which substances or groups of substances further studies are required for a robust assessment. The further development of existing screening models, such as IVIVE (*in vitro* to *in vivo* extrapolation), PBTK (Physiologically Based Toxicokinetic), and mass balance models that are used for the estimation of bioaccumulation in fish and terrestrial vertebrates, is required in order to identify bioaccumulative substances early and reliably. As part of a UBA funded project the application of the screening models and alternative methods are currently critically assessed in order to enable their future use as part of the regulatory bioaccumulation assessment of substances. The limitations of the various screening models (e.g., applicability domain for specific substance properties) and alternative test methods are to be identified.

### 3.01.P-Th147 Consolidation of Log Kow Estimates by Consensus Modelling

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The octanol/water partition coefficient log Kow is a key parameter for assessing the environmental fate and effects of chemicals. The log Kow may be determined experimentally; more often it is calculated. Different methods can sometimes perform better and sometimes worse for different target chemicals, and none of them is consistently superior. Variability may be caused by differences in the applicability domain related to, for example, training set coverage and descriptor relevance. Correct representation of chemical structures needs to consider ionisation, tautomerism, etc.

Comparison of results from different *in silico* methods for log Kow estimation and valid experimental data, e.g., Chemistry Dashboard (US EPA, 2022) including EPI Suite and OPERA, ECHA registered substances (ECHA, 2022), ChemSpider featuring ACD/Labs and ChemAxon (Royal Society of Chemistry, 2022), T.E.S.T. (US EPA, 2020), ChemProp providing Consensus, ReadAcross and LSER models (UFZ Department of Ecological Chemistry, 2022), LSERD (Helmholtz Centre for Environmental Research-UFZ, 2017), OCHEM (eADMET, 2022), indicate the need to consolidate variable, possibly conflicting, results.

Calculations of multiple log Kow for test chemicals including classical POPs, PCB, PAH, siloxanes, flame retardants, PFAS, pesticides, pharmaceuticals, fragrances, biocides, surfactants, UV-filter, plasticizer, antioxidants, phenols, parabens, etc. were analysed to recommend complementary estimation methods. The output is a set of multiple independent methods for log Kow estimations, including guidance on how to use the methods. Consensus modelling, i.e., the mean of multiple log Kow values, including valid experimental data and estimates calculated independently by different methods, such as group contribution, fragmental, atomic values, topological descriptors, or LSER (linear solvation energy relationships), is generally expected to provide the most reliable log Kow predictions. The benefits of the suggested approach are consolidated log Kow that are robust and scientifically credible, reliable, and reproducible, as well as cost efficient.

### 3.01.P-Th148 The Use of *in vivo* and *in silico* Methods for the Bioaccumulation Assessment of Hydrophobic Organic Compounds

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Heterocyclic polyaromatic hydrocarbons substituted with nitrogen, oxygen, and sulfur heteroatoms are likely to pose a major threat to aquatic life based on the indications of persistence and bioaccumulation from screening-level hazard evaluation using *in silico* approaches. Although heterocyclic PAHs are theoretically likely to enter aquatic food web (given the emissions and physicochemical characteristics of these chemicals) and accumulate in higher trophic levels, few laboratory data are available to draw concrete conclusions about bioaccumulation of this chemical class. There are also challenges in testing such hydrophobic and poorly water-soluble chemicals with the current methods, i.e., limitations arise from loss of compounds during testing as well as the lack of standard testing protocols with ethically more acceptable animal alternatives, e.g., crustaceans like *Daphnia magna*. Therefore, we studied the bioaccumulation of several heterocyclic PAHs with *Daphnia magna* using passive dosing method for maintaining a controlled and defined exposure concentration for chemical uptake. We argue that measuring bioaccumulation in daphnids is ethically more acceptable and easier to perform, especially for so-called “difficult compounds”. We also generated data that can be used as proxies of hydrophobicity – octanol-water (log K<sub>OW</sub>) and membrane lipid-water (log K<sub>lip/w</sub>) partition coefficients – which are predictors of bioaccumulation potential. Subsequently, we correlated the bioaccumulation factor (BAF) with log K<sub>OW</sub> and log K<sub>lip/w</sub> to build Quantitative Structure-Activity Relationship (QSAR) models to predict the bioaccumulation potential of heterocyclic PAHs in *Daphnia magna*. Lastly, the relevance of using K<sub>lip/w</sub> over log K<sub>OW</sub> for estimating bioaccumulation/bioconcentration of heterocyclic PAHs was discussed.

### 3.01.P-Th149 Effects of Dietary Selenium on the Freshwater Amphipod, *Hyalella azteca*

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Fish are the most sensitive group of aquatic organisms to chronic toxic effects of selenium (Se), based on reproduction and early life stages of numerous fish species. The US Environmental Protection Agency (USEPA 2016) concluded that Chronic Se toxicity data available for freshwater invertebrates indicate that invertebrates are less sensitive to chronic Se toxicity than fish, but the number of suitable toxicity studies with invertebrates was limited (3 species), and not all these studies included reproductive endpoints. USEPA was interested in development of additional invertebrate toxicity data to confirm that invertebrates are protected by the selenium criterion. We conducted a 28 day feeding study to compare the sensitivity of *Hyalella Azteca* and *Chironimus dilutes*, which indicated that *H. Azteca* were more sensitive. A 42 day chronic feeding study further evaluated the toxicity of Se to the amphipod *H. azteca*. *Hyalella* were fed a combination of selenized yeast and diatoms. Endpoints for the exposures were survival, reproductive success, and Se tissue concentrations. Chronic 42-day exposures with *H. azteca* produced an EC<sub>20</sub> for growth and biomass of 22 ug/g. Effect on reproduction were found as low as 3 µg/g, but these results are preliminary and data are being studied further because the novel approach resulted in less control reproduction than is standard.

### 3.01.P-Th150 Lipid Normalisation in the OECD 305 Dietary Test

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One of the main environmental priorities under the UK and EU REACH Regulations is the assessment and identification of persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substances. A key test method used to investigate bioaccumulation is the OECD 305 Test Guideline (Bioaccumulation in Fish: Aqueous and Dietary Exposure). This test guideline was updated in 2012 to include dietary exposure. To support the update to the test guideline, the OECD together with three lead countries (UK, Germany and the Netherlands) prepared guidance on specific aspects of the dietary accumulation test. This guidance was published by the OECD in July 2017; amongst other aspects, it recommends performing lipid normalisation of the dietary biomagnification factor (BMF) to both the lipid content of the fish and the lipid content of the food for the interpretation of results.

During the latter stages of development of the guidance in 2017, the Chemicals Evaluation and Research Institute, Japan (CERI) presented new research of the effect of different spiked foods in the dietary test. CERI suggested that the dietary BMF should only be standardised for the fish lipid content, not the food lipid content. CERI also suggested that the BMF should be standardised to 5% fish lipid content. The work by CERI is acknowledged in the published OECD guidance, but further analysis of the research in relation to other experimental and theoretical evidence was warranted.

We have reviewed the available experimental and theoretical evidence for lipid normalisation in the dietary accumulation test and this suggests strongly that the growth-corrected and lipid-normalised kinetic BMF value (BMF<sub>kgL</sub>, as defined in the OECD 305 Test Guideline) varies depending on the lipid content of the diet/food used. This can be explained by examining both the differences in the fugacity capacity between the diets of different lipid contents, and differences in the apparent feeding rate, when expressed on a lipid basis. As a result, the lipid normalisation method currently recommended in the OECD 305 test guidance will lead to differences in the growth-corrected and lipid-normalised kinetic BMF obtained when different diets are used. We present recommendations for how the results of dietary accumulation results are best interpreted, with the aim of informing a future update to the OECD guidance document for the OECD 305 test guideline.

### 3.01.P-Th151 Lateral Transport of Pharmaceuticals and Endocrine Disruptors in Riparian Zone Through Food Webs

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Sewage pollution causes the presence of pharmaceuticals (PhAC) and endocrine disrupting compounds (EDCs) in freshwaters worldwide. Like many other emerging contaminants (ECs), PhACs and EDCs can be bioaccumulated in various aquatic organisms. Emergence of aquatic insects connects aquatic and terrestrial food webs in riparian zones causing transfer of pollutants from aquatic to terrestrial food webs. Data on such contaminant flux are very scarce for PhACs and EDCs; for instance, lateral transport through food webs in the riparian zone is still unexplored. With the aim of exploring how food web linkages impact transfer from aquatic to terrestrial ecosystem of PhACs and EDCs we conducted an *in situ* study at a river stretch impacted by the WWTP effluents. More specifically, to study transport of the ECs laterally into the riparian zone, sampling sites on one riverbank were split into three transects – aquatic and two in riparian zone (0-1 m and 1-3 m distance from the waterline, respectively). Sampling involved invertebrates belonging to various levels of food webs, as well as soil, water, biofilm and macrophytes samples. All samples were screened for 123 compounds using ultra-performance liquid chromatography (UPLC) system coupled to a quadrupole time-of-flight mass spectrometer. First results show that 65% of ECs were detected across all samples; however, presence and concentrations of individual compounds vary among sample types. For instance, bisphenol-A (BPA), a chemical widely used in plastics manufacturing, was present in majority of sample types; however, in low concentrations (e.g., up to 0.4 ng/g dry weight in biofilm, up to 1.64 and 2.96 ng/g dry weight in soil and macrophytes, respectively). Furthermore, BPA concentrations in riparian spiders declined with distance from the waterline in the riparian zone, highlighting the importance of trophic transfer of waterborne pollutants into terrestrial habitats, as emerging aquatic insects are known to represent the major food source of riparian spiders. Through providing new insights on food web flux and related lateral extent of the subsidy in the riparian zone, this study will contribute to understanding of the cross-ecosystem flux of PhACs and EDCs.



### **3.01.P-Th152 Quantitative Weight-of-Evidence Approach for Bioaccumulation Assessment of Volatile Methylsiloxanes in Aquatic and Terrestrial Species Using the Bioaccumulation Assessment Tool**

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Bioaccumulation of a chemical is predicted or assessed using different metrics such as bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF), and trophic magnification factor (TMF). The metrics have different criteria for bioaccumulation according to the design of the endpoint. It is not uncommon to obtain conflicting results using the different metrics. We have previously reported an evaluation of the bioaccumulation potential for cyclic and linear volatile methylsiloxanes (VMS: D4, D5, D6, L2, L3, L4, and L5) in aquatic and terrestrial species using the Bioaccumulation Assessment Tool (BAT, ver2.02) that facilitates the evaluation of multiple kinds of bioaccumulation data in a quantitative weight-of-evidence (QWoE) approach. The model assesses bioaccumulation potential with consideration of the relevance and reliability of each available data point. VMS are used as chemical intermediates and some can be found as ingredients in consumer products. As a result, their environmental concentrations are in a wide range in different media including biota, surface water, air, and sediment. Thus, there have been multiple laboratory- and field-based data available such as BCF, BMF and TMFs which as previously reported, were utilized in the tool along with QSAR predicted biotransformation rates and BCFs from the EAS-E Suite (QSARINS, IFS, and OPERA) and the EPA CompTox Chemicals Dashboard. We have now incorporated additional terrestrial data for rats utilizing available pharmacokinetic (PK) and adsorption, distribution, metabolism, elimination (ADME) studies with VMS to assess bioaccumulation potential. The metabolism and elimination rates were determined from these PK/ADME studies and BMF values were calculated utilizing the IVIVE-B model (Lee et al. 2017). BMF values in rats indicated no bioaccumulation for VMS due to relatively fast removal via metabolism and elimination through expired air. When these data were incorporated into BAT, the QWoE conclusion is that dietary uptake of VMS cannot result in bioaccumulation of VMS in both aquatic and terrestrial species.

### **3.01.V Advances in Bioaccumulation Science and Assessment**

#### **3.01.V-01 Bioaccumulation Factor Relationship Analysis with Heavy Metal Concentrations in River Sediments and Dominant Benthic Macroinvertebrates**

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Environmental contaminants have been continuously sink in the aquatic sediments, having detrimental impacts on life of the resident biota, finally disturbed aquatic ecosystems. Heavy metals, one of environmental pollutions from growing population and urbanization, are natural and anthropogenic occurring elements. In the study, we investigated heavy metal distributions in sediments and benthic macroinvertebrate communities to compare heavy metal bioaccumulation by potential contamination sites. To do this, heavy metal concentrations in the body and trace metal concentrations in the sediments of the dominant species at each site were analyzed using an inductively coupled plasma mass spectrometer, and mercury was measured using an atomic absorption spectrometer. As a result of evaluating the degree of heavy metal contamination based on the contamination load index and the geochemical enrichment coefficient, it was found that the lower part of the Suyeong River had the most heavy metal contamination, and in particular, the accumulation of mercury was the most severe. Among the 15 sites, 11 sites satisfied the biomass (Decapod 4 sites, Gastropod 5 sites, Insect class 1 sites, Hairy class 1 sites), the species with the highest concentration of heavy metals in the body was *Chironomus* sp. collected from the main stream of the Yeongsan River. Among the heavy metals, the most frequent accumulations in benthic macroinvertebrates were copper (8 sites) and mercury (8 sites). The copper and arsenic enrichment coefficients showed opposite trends to those representing the health of benthic macroinvertebrate communities. These results suggest that it is necessary to comprehensively assess the degree of contamination of the sediment itself, the degree of accumulation in the organism, and the stability of the community to manage heavy metal pollution in the river ecosystem.

### **3.02 Advances in Exposure Modelling Toward Data-Driven Decision-Making**

#### **3.02.T-01 Plastics in the Environment Using Mass Flow Analysis, Including Degradation, Accumulation, and Environmental Dispersion**

**Anna Schwarz**, Sietske Lensen, Jan Harm Urbanus, Luke Parker and Eline Langeveld, TNO, Netherlands

Plastics are rapidly accumulating in the environment. To assess mitigation strategies, insight in sources and fate of plastics has to be obtained. Plastic products are used and lost among all sectors, in numerous applications, during multiple uses and along different lifetimes. To assess the complex value chain of plastics, a Mass Flow Analysis (MFA) can be used to track plastics in the economy but also to explore losses to the environment. Except for yearly losses, also degradation of plastics in the environment and dispersion of the plastics in the environment are important to assess the plastics' impacts and risks. In this study, a yearly MFA approach was developed for plastics to assess yearly losses of plastics to the environment. The MFA is extended to an Accumulation and Dispersion model (ADM) to simulate global accumulation of plastics in the environment until 2050. Additionally, environmental parameters were developed to assess fate and dispersion in the environment with higher accuracy. The basis of the yearly MFA is based on existing literature and includes potential losses from the full life cycle of the plastic product. Product lifetimes were included in the model to correct for stocks for specific sectors. In both the MFA and ADM, eight

polymer types, seven sectors, 12 environment types, two plastic size classes, and 190 countries were distinguished. The yearly MFA gives interesting insights among multiple dimensions. For microplastics, rubber microplastics from car tyres contribute over 60% of total microplastics lost. Most macroplastics in the environment originate from the packaging sector. According to the ADM, most of the macro- and microplastics will accumulate in terrestrial environments. Only limited micro- and macroplastics lost on land will reach oceanic environments. Quick degradation of specific polymers in environments with UV access will significantly increase the number of microplastics formed. Potential human and biota exposure was assessed, where increased intake depends mostly on microplastic access in indoor air and surface waters. To conclude, the MFA and ADM give insight in quantities of micro- and macroplastic losses to the environment with multiple dimensions. Future scenarios can be modelled for potential impacts on the environment, on biota and human exposure. Also the effect of mitigations, such as material replacements, alternative policies, and clean-up efforts, can be assessed.

### **3.02.T-02 Country-Wide Prediction of (Micro-) Plastics in Freshwaters**

*David Mennekes and Bernd Nowack, Empa – Swiss Federal Laboratories for Material Science and Technology, St. Gallen, Switzerland*

Over the last years, more and more approaches have been introduced to not only model plastic movement in oceans but also in freshwaters. Most microplastics models for freshwaters are based to certain extents on sedimentation modelling or are extracted from sedimentation experiments. The drawback for this model design is the complexity and data requirements which so far restrict the models in their scale of application. Especially, for policymakers relevant national scales are insufficiently covered by existing modelling approaches.

The aim of our work was therefore to develop a microplastic fate model for freshwaters that is able to predict microplastic fate at the scale of whole watersheds or countries. The model was parameterized for Switzerland considering a network of 600,000 river segments and over 200 lakes. Coupling this model to an existing spatially resolved plastic emission model allowed to predict microplastic flows in all river segments and lakes for seven specific polymers (LDPE, HDPE, PP, PVC, PS, EPS, PET). We base our model on existing mechanistic transport models for microplastic but simplified the parameter input in order to create a country-wide model.

Through our work, we are able to predict the contamination of freshwaters by microplastics on the scale of each river section for an entire country. Compared with available catchment scale models, this will provide an even closer look at local sources and sinks of plastics. As one result, we clearly see the influence of lakes which retain microplastics through sedimentation. However, for Switzerland many lakes are located upstream of the major cities which are considered hotspots of microplastic emissions through their waste water treatment plants. Thus, the effect of plastic retention in lakes is lower than expected. Additionally, we differentiate between removed and accumulated plastics as well as plastics in the sediment or water column. Through this, we are able to predict exposure masses on spatial high resolution.

We believe that our work can help to better understand the sources of the global plastic pollution but rises the need for experimental data on plastic transport in the environment. The large-scale understanding of plastic transport processes will provide policy makers with options were to tackle the spread of plastic pollution in the most efficient way.

### **3.02.T-03 Application of Real World Data to Refine Runoff Exposure - Australian Situation**

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Australia has a wide range of environmental conditions in agricultural areas including temperate, subtropical, and tropical. A "one size fits all" approach to exposure modelling does not work and such an approach results in a high failure rate for substances assessed for risk from runoff. The primary reason is that the screening step provides no context in terms of space or time, and runoff risk is therefore assumed to be equal regardless of where in the country application occurs, or at what time of the year.

Real world data are available to address this problem. The data are not held by a single entity and significant data extraction from information held by different organizations has allowed development of necessary real world data sets in the areas of soil types, cropping contribution and slopes in growing regions. Climatic data sets have been developed based on long term rainfall (daily rainfall and intensity) and hydrology characteristics in catchments. This data mining has allowed relationships to be developed and described mathematically for different regions and times of the year.

The climatic data sets are analyzed on a temporal scale (seasonal for temperate regions and monthly for tropical and subtropical regions) and at the highest tier of assessment, cumulative frequency curves for theoretical receiving water concentrations are developed. These curves are developed for each region and at two stream flow levels (25th and 75th percentiles). The methodology allows risk to be described in terms of probability of exceeding allowable concentrations and can result in targeted controls to better address identified risk areas.

The problem facing regulators with the use of such real world data is the size of data sets and the complex relationships that require development. The time required for such refined assessments is a limiting factor, and the scope for human error is large given the high number of different runoff curve numbers, slopes, rainfall values and stream flow rates that need to be included. However, all the algorithms for runoff curves based on soil specific profiles and stream concentration distributions have been successfully coded into a software tool making it possible to undertake such complex assessments rapidly and compare scenarios quickly and consistently. The approach has successfully been applied in Australian regulatory decisions and relied on by industry in preparing submissions.

### 3.02.T-04 Deconstructing Sources of Aquatic Toxicity for the Hundreds of Pesticides in Use, Irrigation Impacts, and Exposure at Scale

*Nicol Parker and Arturo Keller, University of California, Santa Barbara*

Pesticides remain a leading environmental hazard, imperiling aquatic and terrestrial ecosystems. The mitigation of pesticide impacts is hampered by the ability to evaluate their exposure over large extents, the spatiotemporal resolution of use data, the ability to assess cumulative toxicity, and the identification of unique inter and intra-crop irrigation practices on aquatic pesticide exposure. We introduce the Pesticide Mitigation Prioritization Model to advance these four areas within the United States. We describe tool development and apply the tool to evaluate aquatic exposures in California, whose agricultural applicators must provide daily-time step pesticide use reports and whose pesticide use database is recognized as the most comprehensive globally. Additionally, many growers in California must report irrigation methods used; these data are combined with crop-specific evapotranspiration rates, crop rotations, and soil hydrologic properties to predict the effects of irrigation on aquatic exposures of pesticides at scale. California is a valuable study site representing over 400 agricultural application site types. The state's mass of applied pesticides is also 20% of the total used in the United States and 3% of applications worldwide. We apply the tool to address the following questions: 1) How is toxicity distributed among the numerous pesticides in use for diverse aquatic taxa? 2) Are current efforts reducing pesticide toxicity? 3) Does quantifying cumulative toxicity improve our understanding of aquatic exposure at the monthly and annual time steps? 4) Can low water volume irrigation methods render higher acute pesticide concentrations in rainfall-runoff events due to pesticide buildup?

### 3.02.T-05 How Confidently Can Current Computational Models Evaluate Ecological and Human Exposure to the Myriad of Chemicals in Commerce?

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More than 350 000 chemicals and mixtures registered in national and regional chemical inventories are awaiting exposure and risk assessments. Generic mechanistic exposure models are cost-effective and user-friendly tools for this goal. Excellent examples of these models, e.g., EUSES, PROTEX, USEtox, and RAIDAR(ICE), have been used to track ecological and human exposure to thousands of chemicals through multiple routes from multiple sources. In general, the applicability of these models is constrained by (i) the applicability domain of tools used for parameterization of fundamental properties, e.g., quantitative structure-activity relationships (QSARs), (ii) built-in empirical relationships generalized from experimental measurements for parameterization of the rest parameters, and (iii) assumptions adopted by model algorithms, e.g., well-mixing homogenous environmental compartments and steady state. In this presentation, we evaluate the extent to which existing generic mechanistic exposure models apply to 112 000+ chemicals registered in five comprehensive chemical inventories. In brief, using the state-of-the-art QSAR tools, we parameterize the selected models with partitioning and reaction properties to predict exposures of ecological receptors and humans to this myriad of chemicals. We systematically analyze the applicability domains of QSARs, built-in empirical relationships, and model assumptions for these chemicals, whereby we determine the chemicals in these inventories for which these models can make predictions with the highest confidence. Preliminary results indicate that we can be confident in evaluations of exposure for only a small fraction of commercial chemicals using the current computational models. Specifically, violating the assumption of well-mixing in outdoor compartments (notably large water bodies) limits the application of generic, mechanistic exposure models to most chemicals. The applicability domain of empirical relationships limits the application of generic, mechanistic exposure models to more than half of chemicals. The combination of multiple QSAR models greatly expands the applicability domain. Reliably predicting the biodegradation half-life is the current bottleneck of QSAR applications. This presentation, for the first time, systematically informs academia and regulatory agencies on the applicability and uncertainty of the generic, mechanistic exposure models.

### 3.02.P Advances in Exposure Modelling Toward Data-Driven Decision-Making

#### 3.02.P-Tu136 Modelling the Friction of Vehicle Tyres to Estimate Emission of Microplastics

*Johannes Meesters, Jaap Slootweg and Joris T.K. Quik, National Institute for Public Health and the Environment (RIVM), Netherlands*

Understanding the source of microplastics from tyre wear is key for implementing effective mitigation measures and monitoring microplastic emissions. Current country specific procedures to estimate the emission of microplastics from tyre wear are based on outdated measurement data and assume same levels of wear per distance for different car models. Any development in vehicle and tyre design or traffic regulations and road track are as such not accounted for in calculated tyre wear particle (TWP) emissions after the year 2000. A new microplastic emission model for TWPs is presented here that calculates the friction between tyre and track for different vehicles performing different manoeuvres. Model sensitivity analyses indicate vehicle velocity and landscape slope are most determinant in simulating friction work. The abrasion coefficient of tyres that represents the amount of mass abraded per unit of friction work performed (mg/J) is both an important and difficult to collect input parameter. The emission model however can estimate such abrasion coefficients from field data sources that express the level of tyre wear as the amount of mass abraded per driven kilometre (mg/km). As such, the model is used to estimate abrasion coefficients (mg/J) by dividing given wear rates (mg/km) in available data sources with the model outcome for the friction work performed on the respective road track divided by the track's distance (J/km).

Additionally, lab and field measurements are planned in the near future to estimate abrasion coefficients from measured tyre wear and conditions that control the level of friction work. Furthermore, field measurements in which tyre wear abrasion is measured for vehicles performing specific manoeuvres over an outdoor track circuit are used to validate the model script.

It is also planned to set up a database that comprises the relevant vehicle specifications, tyre quality indexes, country traffic regulations and road infrastructures for the period of 2000-2021. The content of the database will serve as input for the TWP emission model routines to calculate the amount of TWPs released per driven kilometre of the vehicles per year.

As such, the model can be used to update country emission factors, quantify the impact of developments in vehicle and tyre design as well as the impact of future policy measures to reduce microplastic emissions from tyre wear. In particular relevant as tyre wear is included in new EURO7 emission criteria.

### **3.02.P-Tu137 Introducing $\mu$ BETR Global, a Global-Scale, Geographically Explicit, Multimedia Microparticle Transport and Fate Model**

*Marianne Seijo<sup>1</sup>, Michael John Whelan<sup>2</sup>, Todd Gouin<sup>3</sup> and Antonia Praetorius<sup>1</sup>, (1)University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics, Netherlands, (2)Centre for Sustainable Resource Extraction, University of Leicester, United Kingdom, (3)TG Environmental Research, United Kingdom*

Owing to the broad spectrum of current and innovative applications and low cost, the production of plastics has drastically increased over the last decades and is still on the rise: Since the 1950s, 8.3 billion tons of plastics have been produced. But the reverse of the medal is that 79% accumulated in landfills and the natural environment. A particular issue is the fragmentation of larger plastic waste into microplastics (MPs), ranging from 5 mm down to 1  $\mu$ m (and < 1  $\mu$ m nanoplastics). The MP (and nanoplastic) size range encompasses different dynamics processes from Newton to Brownian and makes them readily available for ingestion and/or inhalation for a plethora of organisms. Moreover, MPs have been found in all environmental compartments, from soils to air, from freshwaters to oceans. Still, limited mechanistic understanding is available on specific transport pathways and the complex interaction and transformation processes that MPs can undergo in different environmental media over time.

Models play an important role in understanding the emissions, transport, and complex transformation processes of MPs in air, freshwater, soils, sediments, and the oceans, and adequate modelling tools are needed to support exposure and risk assessment. In addition, the particulate nature of MPs and their huge heterogeneity in terms of sources and physicochemical properties require data-intensive modeling approaches, accounting for MP-specific transport and fate processes.

Here we introduce a recently developed global-scale multimedia transport and fate model for MPs. Our  $\mu$ BETR Global model implements the latest developments in MPs mobility and fate on a global scale with a 3D high resolution of 0.5° grid cells (equivalent to 55.0 km) and a real-time scale.  $\mu$ BETR Global is a mechanistic multimedia mass-balance model developed to evaluate short- and long-range transport of MPs and their transformation processes (e.g., weathering, fragmentation, biofouling, and aggregation). The model includes both the role of MPs' physicochemical properties (size, shape, density/composition) and the influence of environmental system parameters on the overall fate. Environmental system parameters are obtained from the Copernicus observation program, the European Centre for Medium-Range Weather Forecasts' (ECMWF's), and Era5 reanalysis data. The focus of this presentation will be a demonstration of the atmospheric transport of MPs within  $\mu$ BETR Global under selected emission scenarios.

### **3.02.P-Tu138 Engagement from the Environmental Chemistry Community is Needed to Protect the Martian Environment**

*John Daniel Hader and Matthew MacLeod, Stockholm University, Sweden*

The production, use, and disposal of anthropogenic chemicals and materials has led to the contamination of the Earth environment, such that a 'planetary boundary' for their safe use is currently exceeded. The introduction of novel entities into natural environments has not stopped at the top of Earth's atmosphere, as an estimated 9979 kg of spacecraft materials from Earth have been transported to the Martian environment. International agreements on the safe and sustainable exploration and exploitation of celestial bodies are limited in scope and legal enforceability. Furthermore, several government and private entities have plans to transport humans to the surface of Mars. Against this backdrop, several frameworks have been proposed for environmental impact assessments of human activities on celestial bodies. Missing from these proposed frameworks, however, is recognition of many of the hard-learned lessons from humans' contamination of the Earth with novel entities, particularly regarding their emissions at the point of material use and subsequent transport to remote regions. We argue that the expertise of the environmental chemistry community should be leveraged to investigate the potential for anthropogenic chemicals to be emitted into the Martian environment, transported regionally and planet-wide, and accumulate into regions with a relatively higher likelihood of harbouring extant life. A logical entry point for the environmental chemistry community into the assessment of novel entities on Mars is the use of chemical fate models. Simple box-models could be used as a starting point to explore the potential for known chemical fate and transport pathways to result in accumulation of chemicals in high-value environmental media (e.g., subsurface water), with further model refinement as workflows develop. Such exercises would likely elucidate knowledge and data gaps related to chemical processes in the Martian environment that could be further explored via experimental work. The environmental toxicology and chemistry community has an immense amount of knowledge regarding local and planetary contamination by anthropogenic chemicals that could aid research focused on protecting the Martian environment. As more governments and other entities set their sights on exploration or exploitation of the Red Planet, it is

imperative that sound interplanetary chemical management is established far in advance of any human expansion deeper into the solar system.

### 3.02.P-Tu139 Application of a Spatially Resolved Model to Refine Exposure Assessment of Down-the-Drain Chemicals in European Rivers

**Chiara Maria Vitale<sup>1</sup>**, Susan A Csiszar<sup>2</sup>, Raghu Vamshi<sup>3</sup>, Brenna Kent<sup>3</sup>, Michaela Koopmans<sup>2</sup>, Diederik Schowanek<sup>1</sup>, Ping Sun<sup>2</sup> and Kathleen McDonough<sup>2</sup>, (1)Procter & Gamble, Belgium, (2)Procter & Gamble, (3)Waterborne Environmental, Inc.

A spatially resolved model was developed for Europe that predicts the fate and transport of chemicals disposed down-the-drain and estimates aquatic concentration distributions (i.e., percentiles) in receiving waters. The model uses chemical specific information (per-capita emissions, wastewater treatment plant (WWTP) removal, in-river decay) and spatially explicit data on WWTP infrastructure, population served and river basin hydrology, to simulate water and chemical routing and predict in-stream concentrations. This modelling framework, built upon the iSTREEM® framework, which contains geographic datasets for USA, China, and Japan, was implemented for European countries, using spatially refined publicly available datasets. The USDA Curve Number method was used to generate spatially resolved mean river flows for use in the model. Chemical case studies were run for common surfactants used in cleaning products with down-the-drain emissions (i.e., linear alkyl benzene sulphonates (LAS); alcohol ethoxysulphates (AES); alcohol sulfates (AS)) to evaluate model performance. Predicted environmental concentrations (PECs) in rivers were compared to monitoring data, as well as simulations performed by EUSES predictions, as a comparison. Results suggest that the spatially resolved Europe modeled PECs were predictive while being conservative when compared to monitoring data. The modeled PECs also reproduced the spatial variability of monitored concentrations with agreements generally greater than an  $R^2$  of 0.9. Additionally, EUSES estimates were also conservative when compared to the monitoring data and the spatially resolved model can be used to further understand differences between these results and to perform refined exposure assessments for down-the-drain chemicals.

### 3.02.P-Tu140 Automated Classification of the German Soil Map (BUEK 200) into FOOTPRINT Soil Types and Parameterization in MACRO

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The FOOTPRINT Soil Type (FST) system has been derived during the FOOTPRINT project (2006-2009) to facilitate spatially distributed pesticide fate modelling at national or EU scale. The basic idea of this approach is to classify the soil typological units (STUs) of a national or European soil database into a limited number of soil types (FSTs) in order to reduce the number of unique soil-climate combinations for the later numerically expensive simulations with a pesticide fate model. The FST code consists of a hydrological class (the FOOTPRINT Hydrologic Group), a topsoil and a subsoil texture code, and an organic matter profile code. The FST system is model-independent, but complete parameterization methodologies for MACRO and PRZM were established during FOOTPRINT. In this study we i) translated the latest version of the German soil map 1:200,000 (BUEK200) into FSTs, ii) derived representative profiles for all FSTs with arable land use, and iii) parameterized these representative profiles in MACRO. The 3648 STUs with arable land use in the BUEK200 were classified into 226 FSTs. Area proportions covered by the different FSTs are highly skewed: The 13 FSTs with the largest areas already cover 50% of the total arable land. The hydrological class of each FST indicates whether artificial drainage is needed to allow arable land use and a map of potential drained arable land was derived for Germany accordingly. A representative soil profile was established for every FST by depth-based averaging over all soil profiles belonging to the same FST. The plausibility of the representative FST profiles and their MACRO parameterization was checked with water balance simulations. The present case study for the BUEK200 soil database demonstrates the potential of the FST system for spatially distributed fate modelling at large scale based on national soil databases.

### 3.02.P-Tu141 Using Remote Sensing Methods to Characterize Grassland Landscapes for Scenario Development and Biodiversity Assessment

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Grasslands support essential biodiversity and ecosystem services, and their resilience is threatened by climate change and degradation through anthropogenic land use changes. Remotely sensed satellite imagery has previously been utilized to characterize the intensity and usage of grasslands over time and broad geographic areas. We used synthetic aperture radar and optical multispectral imagery with repeat images captured every six and five days (respectively) to identify grassland changes at a high temporal resolution, as highly managed grasslands may be cut multiple times per season (March – October). Time series images were used to characterize grassland type, cutting frequency, and use intensity for over 6000 grassland parcels in Germany between 2018 and 2021. Training data were collected for grassland types including; silage, meadow (hay), pasture, and natural grassland. Spectral indices related to vegetation were derived across grassland parcels to create annual metrics. Thresholding methods were applied to annual time series of Sentinel-1 and Sentinel-2 to detect cutting events within each grassland patch. Results from the two methods were combined to improve cut detection confidence. A random forest modeling approach was used to classify the grassland type and use intensity. The case study area studied was dominated by silage grassland type, and results estimated that 88% of all grassland parcels were cut three or more times per year. The final statistics provide a suite of metrics that may be useful for assessing the quality of insect habitat (e.g., first grassland cutting of the year), habitat connectivity, and investigating which grassland management practices best support biodiversity. Comparable remote sensing approaches have been used to develop landscape scenarios for honey bee (*Apis mellifera*) risk assessment, selection, and characterization of habitat for wood mouse (*Apodemus sylvaticus*) population models supporting regulatory pesticide risk assessment, and within other studies

to characterize insect habitat and biodiversity. Outlook of future activities incorporating these technologies includes fusion of multiscale data sources (e.g., UAVs, satellites, *in-situ* field observations), and diverse sensor platforms applied to a wide variety of ecological investigations.

### **3.02.P-Tu142 Comparing the Sensitivity of Predicted Environmental Concentrations of Pharmaceuticals Using Empirical and Quantitative Structure-Activity Relationship (QSAR)-Derived Physicochemical Parameters**

**Jeff Rominger**, Tim Verslycke and Wasfia Hoque, *Gradient, Boston, Massachusetts*

Environmental exposure assessments of active pharmaceutical ingredients (APIs) require information about API behavior in environmental media. Upon release into the environment, APIs can be present in wastewater, biosolids, surface water, groundwater, sediment, and soil, differentially partitioning among these media according to their physicochemical properties. APIs also degrade following release through various abiotic and biotic processes, thereby reducing their environmental concentrations over time. Environmental exposure assessments often estimate predicted environmental concentrations (PECs) in an environmental medium to assess potential human or ecological exposures. However, information on API partitioning and degradation is often not available for many APIs, presenting an important obstacle in environmental exposure assessments.

In the absence of empirical data, quantitative structure-activity relationship (QSAR) data are often used as alternatives to support environmental exposure assessments. QSARs rely on predictive relationships between a chemical structure and its physicochemical or environmental properties. QSARs have wide use in chemistry, biology, toxicology, and environmental applications. However, there is uncertainty associated with the use of nonempirical data to inform exposure assessments and the magnitude of this uncertainty is often unknown.

In this study, we calculated the sensitivity of exposure estimates to the use of QSARs by comparing the difference between PECs calculated using QSARs versus those calculated using empirical API parameters. To perform this study, we employed a recently developed environmental fate tool that incorporates existing exposure assumptions from applicable regulatory guidance (e.g., EMA, ECHA, USEPA) and can be adjusted to account for local hydrogeology as well as exposure pathway (e.g., land application of wastewater biosolids). The results for a suite of APIs show that PECs can be sensitive to physicochemical parameters in certain settings, and that, in the absence of empirical data, using a range of QSAR-derived parameters in a sensitivity analysis framework in environmental exposure assessments can help in evaluating uncertainties of the results.

### **3.02.P-Tu143 Tracking a Chemical's Journey from Production Line to Body Burden Using the PROduction-To-EXposure (PROTEX) Model**

**Li Li**<sup>1</sup>, Dingsheng Li<sup>1</sup>, Alessandro Sangion<sup>2</sup>, Zhizhen Zhang<sup>1</sup>, Christopher Hoang<sup>3</sup>, Jon A. Arnot<sup>2</sup> and Frank Wania<sup>3</sup>, (1)School of Public Health, University of Nevada, Reno, (2)Arnot Research and Consulting Inc. (ARC), Canada, (3)Department of Physical and Environmental Sciences, University of Toronto, Canada

Human and ecological exposures to synthetic chemicals involve chemical emissions from multiple lifecycle sources, multimedia environments at multiple scales, multiple sources of exposure, and multiple pathways of intake and elimination. These components are governed by the complicated interactions between chemical properties, environmental conditions, and human and ecological characteristics. Such multidimensionality and complexity warrant a systematic, mechanistic perspective in exposure modeling and necessitate a holistic approach to track a chemical's journey from the production line to body burden. In this presentation, we introduce a state-of-the-science model named PROduction-To-EXposure (PROTEX), which supports characterizing the entire continuum from chemical production, lifecycle multisource emissions, multiscale multimedia environmental fate and transport, and multipathway exposure, to the presence in humans and other organisms. PROTEX builds on theories of substance flow analysis, multimedia fate and transport, bioaccumulation, and exposure and toxicokinetics, and it integrates mechanistic descriptions of various physical, chemical, biological, and socioeconomic processes. Its mechanistic nature allows PROTEX to be parameterized for a wide range of chemical substances (both neutral and ionizable chemicals) in a wide range of regional environments (e.g., the subtropical US, the Canadian side of the Lake Ontario basin, central China, and the western Baltic drainage basin) for a wide range of populations (Whites, Blacks, Asians, etc.). When supplied with time-variant production or use data, PROTEX provides dynamic time-dependent estimates, allowing users to reconstruct the long-term history of chemical contamination. By considering interindividual variabilities in human anthropometrics, behavior, and physiology, PROTEX additionally supports predicting the distribution of chemical exposure within a population. Outputs from PROTEX support science-based risk assessment and decision-making. The presentation serves as a knowledge hub for PROTEX's rationale and mechanism, applications and case studies, and user tips.

### **3.02.P-Tu144 Modelling the Effects of Microplastic on the Fate of Persistent Organic Pollutants and Their Entry into the Base of Aquatic Food Webs**

**Brendan Eoin Hickie**, *Trent University, Canada*

Concerns about microplastic pollutants in aquatic ecosystems have been increasing exponentially over the last 15 years. One of the leading concerns with microplastics is that they absorb hydrophobic persistent organic pollutants (POPs) from the environment and, upon ingestion, may enhance the bioaccumulation of these pollutants. This however has yet to be demonstrated in either field or laboratory studies using environmentally relevant concentrations of microplastic particles and particulate organic matter (POM) including algae. To explore this, microplastics were added into the well-established fugacity-based Quantitative Water, Air, Sediment Interaction (QWASI) Model to examine whether their presence would affect the overall fate of POPs in aquatic ecosystems. We also added a bioenergetics and kinetics-based submodel for herbivorous zooplankton to estimate

microplastic ingestion rates and any associated POPs accumulation at environmentally realistic concentrations of microplastics, algae, and other POM. The model shows that even the highest current environmental microplastic concentrations have virtually no effect on the overall aquatic fate of POPs spanning a wide range of hydrophobicity, nor do they contribute to POPs accumulation by zooplankton. Our results show that POPs accumulation by zooplankton is dominated by direct partitioning from water, while any accumulation from ingested particles would be mainly from algae rather than microplastics owing to their much greater abundance, and higher bioavailability of algae-associated POPs. The model also provides estimates of density-dependent microplastic ingestion rates which may be useful for addressing other concerns about microplastics. Our research also highlights the need for greater consistency in sampling of microplastic abundance and characterization of their particle size distribution, especially for particles <100 µm.

### **3.02.P-Tu145 Radon Exposure in Indoor and Outdoor Environments for Subpopulation Groups Using Monte-Carlo Simulations**

*Soo yong Lee, Sang hun Im and Hee Seok Kim, Department of Nano, Chemical and Biological Engineering, Seo Kyeong University, Korea, Republic of (South)*

Although monitoring of radon has been extensively implemented throughout South Korea, the risk assessment was mainly limited to indoor environments such as schools, workplaces, and multiuse facilities, and was evaluated separately. In this study, the differences in radon exposure according to the life cycle (0, 1-6, 7-13, 14-18, 19-24, 25-39, 40-64, and 65 years age or older) were evaluated using Monte-Carlo simulation, comprehensively considering various indoor and outdoor environments, time-activity patterns, variations in radon concentrations, and dwelling type. The distribution and representative values of radon concentration by microenvironments were confirmed through the Anders-Darling test, and a uniform distribution was applied in case of uncertainty. The effective dose (DT) ranged from  $2.35 \pm 2.14$  to  $2.81 \pm 3.03$  mSv/y in single-detached houses, and the DT in apartment houses ranged from  $1.45 \pm 9.78$  to  $2.15 \pm 1.27$  mSv/y. Comparing the levels recommended by EPA, WHO, and ICRP with the top 5% values of this study, the results of the population groups aged 0, 1-6, 7-13, and 14-18 in the type of detached houses were exceeded. The populations that spend a lot of time in homes and/or schools (elementary, middle, and high schools) with relatively high levels of concentration are assessed to be relatively more vulnerable to radon exposure.

### **3.02.P-Tu146 Great Britain-Specific Environmental Exposure Scenarios for Chemicals in EUSES**

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The primary environmental exposure model used in UK REACH is EUSES, which was developed more than 20 years ago to serve the purposes of the European Union (EU). A previous scoping study for the Environment Agency in 2018 identified several potential changes and updates to EUSES, including targeted parameterisation to make the outputs more useful and relevant to UK REACH stakeholders, particularly regulatory agencies.

Within this current project, key environmental parameters within EUSES were identified which could be altered to be more specific to Great Britain (GB). Key parameters that are altered included temperature, rainfall, wind speed, soil parameters, sewage sludge spreading assumptions, dilution factors to rivers and coastal areas, size of the regional scale, and food intake for humans exposed via the environment. Parameters that may require amendment to reflect future projected climate change scenarios in GB were identified. The impact of these parameters will be assessed for several time periods.

GB-specific data have been collected for temperature, wind speed, and rainfall with average values over the last 20 years as selected values. The impact of future climate change predictions on these values has also been considered. Data on GB-specific soil parameters and sewage sludge application on soil have also been collected. A catchment area containing an industrial area and a large population was selected as the regional scale. The continental scale was altered to GB dimensions.

A sensitivity analysis has been conducted to identify GB-specific parameters which have the most impact on the predicted environmental concentrations (PECs) derived in the exposure model compared to the standard EUSES model. This was illustrated using a small number of example substances with variable physicochemical and environmental fate properties. The differences between the standard and GB-parameters are discussed and the potential for use of a GB-adapted EUSES model under UK REACH is considered.

### **3.02.P-Tu147 SimpleBox in R**

*Jaap Slootweg, Joris T.K. Quik and Johannes Meesters, National Institute for Public Health and the Environment (RIVM), Netherlands*

SimpleBox is a well-known “fate model” that supports (European) chemical regulation (e.g., REACH). The model comprises of boxes, which are combinations of compartments at nested geographical scales. The original version is implemented in excel, which allows easy adaptation for specific use, for instance a version for nanomaterials. But this implementation has several disadvantages, most importantly version control of both the data and the model and limiting the use to steady state. We present a novel implementation of SimpleBox in R that overcomes the mentioned disadvantages and has other advantages as a bonus.

The design of the R version is object-oriented, using the R6 package, with methods disclosing and extending the data, enabling a single codebase for general and specific use, and easy integration with R-shiny as user-interface for users not specialized in programming. The basis of SimpleBox in R is the easy and transparent way the process algorithms are assembled in the R6 object oriented classes: Core, State, Emission, and CalcGraph.

The “Core” class is the most prominent object and handles the internal data, others are “injected” in it. The State class generates the scenario (boxes of a compartment in a scale and their coherence). The Emission class defines the interface for emissions. Other classes inherit from the so-called CalcGraph class. Instances of these subclasses are initiated with a (simple) function as argument. These functions have atomic arguments, are automatically called whenever needed, and are provided with the arguments corresponding to the data in the Core. Subclasses are for (1) defining the velocity of the transfer processes, (2) calculating intermediate variables for boxes stored in the Core for future use, (3) calculating fluxes associated with a transfer process, and (4) solving the set of first-order differential equations; either for steady state, dynamic responses, or sensitivity analyses. Functions for the CalcGraph classes to reproduce the current version are available but can be changed easily. The steps to define a scenario, calculate results and plot them in graphs are kept in R scripts which are concise and clear due to the use of the R6 classes. The logic of your SimpleBox application can thus easily be defined, documented, shared and archived. In this script you can initiate a Core with a State object and an Emission object both extended for your scenario. SimpleBox in R will be made available on github

### 3.02.P-Tu148 Evaluation of Models Coupled with Measured Data for Determining Organic Carbon-Water Partition Coefficients

*Jaeshin Kim, Gary Kozerski and Jeremy Durham, Dow Chemical Company, Midland*

The organic carbon-water partition coefficient ( $K_{OC}$ ) plays an important role in determining the fate and distribution of chemicals using environmental multimedia models. The sorption process into particulate organic matter in soils and sediments can compete with other fate processes such as volatilization, degradation, advection, sedimentation, etc. Especially for volatile methylsiloxanes (VMS), many published studies suggested selecting accurate values to predict the environmental behaviors at environmentally relevant conditions. Thus,  $K_{OC}$  values at different temperatures relevant to real world conditions are needed although most  $K_{OC}$  values have been measured at 25 °C or at room temperature. Various methods have been employed to measure or predict equilibrium concentrations of target chemicals in both phases of particulate organic carbon and water. It would be preferable to measure equilibrium concentrations in both phases at different temperatures (i.e., a batch equilibrium method), but more resources are required for the method than other indirect methods that may be used to predict equilibrium concentrations. In the latter indirect methods, only water concentrations are measured over a testing period of desorption from the chemical-laden organic phase. In this case, chemical concentrations in organic carbon phase are predicted. In another case, chemical concentrations in both water and organic phases are predicted by measuring gas-phase concentrations via volatilization from water, similar to a purge-and-trap method. The prediction can be done using a form of modeling with assumptions. We have developed a model to predict equilibrium concentrations for lab experimental conditions and performed sensitivity analysis for input parameters at different temperatures. The model suggested that desorption rate from VMS-laden organic carbon phase, mass transfer coefficient at the air-water interface, and the fraction of loss via degradation and irreversible sorption must be well characterized to predict  $K_{OC}$  values. It is also noted that the methods that predict an equilibrium concentration(s) are less precise and more variable than a batch equilibrium method. When measured data from an indirect method were optimized with model parameters, model equifinality was possible where different  $K_{OC}$  values with distinct model parameters would be obtained.

### 3.02.P-Tu149 Environmental Fate Modeling of Volatile Methylsiloxanes and Degradation Products

*Jaeshin Kim, Gary Kozerski and Debra McNett, Dow Chemical Company, Midland*

Various environmental multimedia models have been developed and applied for volatile methylsiloxanes (VMS). The models have been crucial in understanding the behavior of VMS in the environment and developing tools to determine persistence, long-range transport, and bioaccumulation. Among many input parameters for the models, degradation rates are key parameters for the prediction of the fate of VMS as parent compounds. The main degradation pathways are atmospheric oxidation by hydroxyl radicals, hydrolysis in water, and surface-catalyzed hydrolysis in soil. As a result of degradation process, VMS can be transformed into a series of degradation products at different rates of formation. Since degradation products have different physicochemical properties from VMS, their formation (or emission) and distribution in the environmental media would also be different. Since degradation can be reversible as in the case of aqueous hydrolysis, it is reasonable to model a parent compound and its degradation products simultaneously so that we may develop a holistic understanding of environmental fate of VMS. In this study, we focused on the fate of VMS and degradation products in aquatic systems. Hydrolysis kinetics were evaluated for several degradation products (i.e., dimethylsiloxane- $\alpha,\omega$ -diols and silanols) and their hydrolysis rate constants. In addition, main partition coefficients (such as  $K_{OW}$ ,  $K_{AW}$ , and  $K_{OA}$ ) of VMS and degradation products were estimated using reliable quantitative structure-property relationships. We developed a fugacity-based multimedia model where mass balance equations for the multiple chemical species were solved simultaneously. We will discuss model results of mass distribution and transport as well as environmental concentrations for VMS and degradation products.

### 3.02.P-Tu150 Go-Phytodron Project. Validation and Safety of Unmanned Aerial Spraying Systems (UASS) in Forest and Crops. Preliminary Drift Results

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The European Commission’s Farm to Fork strategy aims to reduce the use of chemical plant protection products (PPPs) in the EU



by 50% over the next decade. This initiative pursues facilitating the adoption of new technologies of application able to improve effectiveness and competitiveness to ensure the sustainability of agricultural systems under the principles of precision agriculture.

Over the last years, many research institutes, chemical companies, and other entities have been looking for development of practical and novel solutions to spray applications problems and integration of new technologies into spray processes for application of plant protection products (PPPs). Among these technologies, UASS (Unmanned Aerial Spraying Systems) provide an opportunity to introduce precision agriculture techniques, such as spot spraying, becoming a promising tool with potential to increase spatial resolution and, consequently, to optimize the amount of product applied, towards a friendly environmental agriculture.

GO\_PhytoDron project (<https://gophytodron.es/>), co-funded by Spanish Ministry of Agriculture and European Agricultural Fund for Rural Development (EAFRD), aims to promote the use of UASS as a precise and safe tool for the application of PPPs seeking to advance in its regulatory framework and establishing scenarios of use.

The project includes field trials to assess potential drift (ISO 22866:2005) on target depositions (ISO 22522:2007), product efficacy, and humans' health exposure to chemicals of UASS in comparison to conventional ground spraying.

The selected crops, olive trees, citrus, and vineyards, are representative of the diversity of three-dimensional crops cultivated in Spain. Pine trees were also chosen to validate the use of this technology in plant health management in cultivated forest. Trials took place across the Spanish territory, covering different climates and conditions representative of the heterogeneity of agricultural systems.

In this work we compare the preliminary results of drift potential (airborne and sedimenting) an from UASS and conventional ground sprayers, together with drift values used currently in the aquatic and terrestrial risk assessment in the EU framework of approval of active substances of PPP, with the aim of developing of specific scenarios for UASS treatments in the future.

### 3.02.P-Tu151 Dissipation of 11 Micropollutants in Soils

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Various micropollutants may enter soils due to the application of treated wastewater or biosolids from wastewater treatment plants. Their leakage from soils and their uptake by plants is controlled mainly by sorption and degradation in soils. Standard laboratory batch degradation experiments were performed using soil samples from the top horizons of three different soil types (Arenosol, Cambisol, and Chernozem) and eleven micropollutants. The wet soil was preincubated in the dark at a constant temperature of 20 °C to guarantee optimal microbial conditions. Next, a solution of one compound was added to achieve similar compound loads per dry soil unit ( $1 \mu\text{g g}^{-1}$ ), and incubation continued. Three samples for each soil with each compound were analyzed immediately, 1, 2, 5, 12, 23, 40, 61, and 90 days after the compound's application to find concentrations of the remaining compound in soils. The first-order and second-order kinetic expressions were next used to fit experimental data points. The first-order equation correctly described the dissipation trends, but the second-order equation was more suitable in a few cases. The highest dissipation half-lives were observed for memantine ( $212 \pm 78$  days), followed by venlafaxine ( $203 \pm 8$  days), atorvastatin ( $14 \pm 5$  days), telmisartan ( $98 \pm 72$  days), sertraline ( $50 \pm 26$  days), lamotrigine ( $48 \pm 15$  days), 2-phenyl benzimidazole-5-sulfonic acid ( $44 \pm 26$  days), 1-methyl-1H-benzotriazole ( $38 \pm 13$  days), valsartan ( $10 \pm 7$  days), triclosan ( $8 \pm 0.4$  days), and bisphenol S ( $4 \pm 1$  days). However, for different micropollutants, the same trends of their dissipation with respect to the tested soils were not observed. Data from this study will be coupled with the maps of the Freundlich sorption coefficients, which were derived for agricultural soils in the Czech Republic, to assess the potential threat associated with using treated wastewater for irrigation or the application of sewage sludge onto soils as a source of nutrients. This information will be used to improve the monitoring of groundwater quality within the Czech Republic, to identify areas unsuitable for broader utilization of these two resources, or to propose their safe application onto agricultural soils.

### 3.02.V Advances in Exposure Modelling Toward Data-Driven Decision-Making

#### 3.02.V-01 Automated Tracking of Changes in Agricultural Land Use by Means of Deep Learning

**Su Yeong Park** and **Magnus Wang**, WSC Scientific GmbH, Germany

Field margins play a crucial role in sustaining biodiversity in intensively managed agricultural lands. In Germany, agricultural land use areas are reported to be continuously decreasing since reunification, changing the structure of arable landscapes including the field margin. However, while the land use changes are monitored over a long period of time through survey and remote sensing technologies, tracking structural changes or field margin changes was rare.

The contribution aims to examine the impact of land use changes on field margins and agricultural landscape structures (e.g., heterogeneity) in Germany for the last decade. For the analysis, the deep-learning based image processing method ALDiS is applied to historical and current high-resolution aerial photographs and quantified structural differences, especially in field edge areas. The selection of landscapes was based on the agricultural area statistics from the German state office, by choosing the

districts with relatively high agricultural land use changes both for the increase and decrease. This contribution will provide examples of how field margin and structure are driven by land use changes, addressing its role as one of the stressors in the future pesticide risk assessment and management.

### **3.03.P Analysis and Evaluation of Efficacy, Stability, Degradation, Release of Biocides From Building Materials and Their Effect on Terrestrial Ecosystems**

#### **3.03.P-Th153 Point and Nonpoint Sources of Urban Biocides in Sediments from Stormwater Infiltration Facilities**

**Felicia Linke<sup>1,2</sup>, Omoyemi Edun<sup>3</sup>, Tobias Junginger<sup>4</sup>, Sylvain Payraudeau<sup>4</sup>, Gwenaél Imfeld<sup>4</sup>, Frank Preusser<sup>2</sup> and Jens Lange<sup>3</sup>,** (1)Chair of Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg, Germany, (2)Institute of Earth and Environmental Sciences, Faculty of Environment and Natural Resources, University of Freiburg, Germany, (3)Chair of Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Germany, (4)Institut Terre et Environnement de Strasbourg (ITEST), University of Strasbourg, France

Stormwater infiltration facilities (SIF) are part of sustainable stormwater management which helps infiltrate stormwater locally and reduce urbanization impacts on groundwater recharge. SIF collect urban runoff containing contaminants such as biocides, which are frequently used as film preservatives on building facades and washed off during wind-driven rain. Previous studies found biocides in stormwater, wastewater, standing water in SIF, and groundwater. However, only a few studies investigated biocide occurrence, distribution, and behaviour in SIF sediments. While input types of biocides have not been characterized yet, we distinguish between point sources of biocides with high emissions from single locations and nonpoint sources from diffuse entry of biocides. Here we quantified three widely used biocides (diuron, octylisothiazolinone - OIT, terbutryn) in paints and renders and selected transformation products in sediments of 46 SIF in two cities of Germany and France. SIF ages varied from 2 to 19 years. Biocide occurrence in SIF sediments differed for the respective substances. Terbutryn was detected and/or quantified at low concentrations (max.  $1.7 \text{ ng g}^{-1}$ ) in 36 out of 46 SIF. This suggests that terbutryn, which is relatively stable ( $t_{1/2}$  90d in soil), may be used as a marker for nonpoint urban biocide emission. Furthermore, recently built SIF showed higher terbutryn concentrations in their sediments than older SIF. This is likely due to a higher biocide load from connected buildings with recently painted facades. Worthy of note, soil characteristics of SIF sediments, such as organic carbon content and clay content, did not correlate with terbutryn concentrations. Diuron and OIT were only found in one SIF, but in very high concentrations ( $168$  and  $58 \text{ ng g}^{-1}$ , respectively), which suggests recent point source emission from, e.g., repair works or construction site. Five occasional detections of acetochlor (max.  $3.2 \text{ ng g}^{-1}$ ), an herbicide whose use is banned in agriculture, supported the occurrence of point source emission. Detection of diuron, OIT, and acetochlor confirmed rare but high biocide inputs in urban SIF. Overall, low biocide accumulation in SIF soils in both cities but punctual inputs in specific SIS invite future studies to extend our approach to other micropollutants and across the facade-soil-groundwater continuum.

#### **3.03.P-Th154 Simulation and Experimental Validation of Distribution of Biocides in Soil Considering a Realistic Emission from Building Façades**

**Nadine Kiefer<sup>1</sup>, Judith Klein<sup>2</sup>, Michael Klein<sup>2</sup>, Mirko Rohr<sup>3</sup>, Michael Burkhardt<sup>4</sup> and Stefan Kalkhof<sup>5</sup>,** (1)Department for Bioanalysis, Coburg University of Applied Sciences and Arts, Coburg, Germany, (2)Fraunhofer IME - Institute for Molecular Biology and Applied Ecology, Schmallingenberg, Germany, (3)Institute of Environmental and Process Engineering (UMEC), Eastern Switzerland University of Applied Sciences (OST), Rapperswil, Switzerland, (4)Eastern Switzerland University of Applied Sciences (OST), Rapperswil, Switzerland, (5)Institute for Bioanalysis, HAW Coburg, Germany

Biocides are used in building materials to protect the building from microbial colonisation and resulting discoloration or even decomposition. However, these biocides are gradually washed out and are emitted into the soil near the building. To obtain a realistic estimate of the toxicity of façade runoff to the soil environment, a predictive tool for risk assessment is definitely required. Thus, the aim of our study is on the one hand to realistically simulate the resulting concentration of various biocides in different soil depths over the lifetime of the building and on the other hand to verify this simulation experimentally.

For the simulation using PELMO three different scenarios for the generation of the biocide emission data were used: (i) experimentally determined in a natural weathering experiment, (ii) based on this natural weathering experiment the emission curve has been modelled in smaller time steps with the software COMELAM and (iii) the same paint/render formulations were tested in the lab using the DIN EN 16105 immersion test and were utilized as emission curve for the simulation. All emission curves were normalized concerning the soil/façade ratio based on the model house described in the OECD Guideline for product type 10. For the experimental validation a set-up has been developed and applied to investigate ten sampling time points at six different soil depths within three months. During the experiment, 3 months of the modelled intake scenario was used experimentally to compare the predicted concentrations with experimental data. Both experimental assessments of biocide leaching and of biocide distribution in soil require time-consuming and costly studies. Therefore, there is an urgent need for reliable simulation software for risk assessment of biocides from building materials. Due to the many material combinations, house geometries, different local weather influences and material and substance related leaching behaviour, estimating application concentrations for further simulations with tools like PELMO is difficult. COMELAM is a solution that provides values comparable to natural weathering experiments even for an artificial immersion test and is able to capture the entire weathering parameters in their complexity. The combination of these two tools with experimentally determined adsorption and degradation values were experimentally valuated for could thus may can reduce or even replace the number of required free weathering tests.

### 3.03.P-Th155 Simulations with PELMO (Pesticide Leaching Model) Describing Biocide Leaching in Urban Soils

**Dimitrios Alexander Skodras**, Judith Klein and Michael Klein, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology, Schmallingenberg, Germany

The original FOCUS (Forum for Co-ordination of pesticide fate models and their Use) models describe agricultural areas that represent realistic worst case scenarios and take into account all relevant target crops, topography, soil, and climate. The locations are geographically distributed throughout the EU (European Union) and show a wide range of temperatures and precipitation. In this study, the FOCUS models shall be used for biocides leaching from buildings. In this case, the application pattern is described by façade leaching data and to adequately reflect the dynamics of façade leaching, an hourly resolution is used. Compared to the classic FOCUS soil profiles, urban soils contain high stone contents (coarse soil). It is necessary to adapt the soil profiles to account for the higher stone content in the soil profile. Due to the stone content, urban soils have an extremely low ability to store water. This affects the infiltration behaviour. The transport of water (and possibly of biocides) is accelerated. Furthermore, there is evidence for fast transport (preferential flow) in urban soil.

### 3.03.P-Th156 Determination of Fumagillin Residue in Honey by Liquid Chromatography Coupled with Tandem Mass Spectrometry

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Fumagillin is used as an antibiotic in apiculture to control nosema disease. To investigate the fumagillin residue in honey after feeding the bees with sugar syrup, analytical methods for fumagillin in feeding sugar syrup and honey were both developed. The analytical method validation of fumagillin in sugar syrup was conducted to assess the specificity, system suitability, linearity, accuracy, precision, carryover effect, and various stabilities using liquid chromatography. Meanwhile, the analytical method validation of fumagillin in honey was also conducted to assess the selectivity, linearity, accuracy, and precision using the liquid chromatography coupled with triple quadrupole tandem mass spectrometry.

The concentrations of calibration standards ranged from 1 to 10 µg/mL for sugar syrup and ranged from 5 to 250 ng/mL for honey. The correlation coefficients ( $R^2$ ) of the calibration curves were all higher than 0.999. The accuracy of the analytical method was checked by recovery results, and the precision was estimated by the relative standard deviations (RSDs) of three replicate samples. As the results, the mean accuracies ranged from 100.4–102.7% for sugar syrup and ranged from 99.4–107.0% for honey, while the precisions were within 1.1% for sugar syrup and ranged from 3.6–13.8% for honey. In both analytical conditions, there was no interference peak to affect the quantification of fumagillin.

Especially, fumagillin in sugar syrup was confirmed to be stable at room temperature (7 days) and refrigeration (14 days), but it could be photolysis in one day. Thus, all the samples were stored and analyzed without light to avoid the photolysis of fumagillin. Consequently, these results are within the acceptable ranges specified in the guidelines for analytical method validation. Therefore, both developed analytical methods could be applied to determine fumagillin residue in sugar syrup and honey.

### 3.03.P-Th157 Method Validation Comparison of Liquid-Liquid Extraction and QuEChERS Methods to Quantify Clopidol in Chicken Tissues

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Clopidol is a feed additive that is used worldwide to control coccidiosis in chickens. To investigate the clopidol residue in chicken tissues, analytical methods for clopidol in chicken tissues were developed. So far, liquid-liquid extraction (LLE) and QuEChERS methods were mainly used pretreatment methods to determine clopidol in various biota samples. Thus, in order to establish an optimized method for clopidol analysis, both pretreatment methods were conducted and compared. The analytical method was validated with respect to parameters such as selectivity, linearity, accuracy, and precision. The accuracy and precision were also evaluated based on three replicate samples at four different spiking concentrations.

As the results, the concentrations of calibration standards ranged from 0.5 to 50 ng/mL, and the correlation coefficients ( $R^2$ ) of the calibration curves were all higher than 0.99 in both compared methods. The accuracy of the pretreatment method was evaluated by recovery results, and the precision was estimated by the relative standard deviations (RSDs). The mean accuracies were 84.0–90.8% for LLE and 86.5–89.0% for QuEChERS, while the RSDs ranged from 1.8 to 12.6% for LLE and ranged from 0.9 to 3.2% for QuEChERS, respectively. Additionally, there was no interference peak to affect the quantification of clopidol in both pretreatment methods. Consequently, these results are within the acceptable ranges specified in the guidelines for analytical method validation.

Both LLE and QuEChERS methods showed similar and satisfied accuracy and precision results in our current study. However, besides QuEChERS having the advantage of a faster and easier pretreatment process than LLE, both methods were suitable for quantifying clopidol in chicken tissues. Therefore, both pretreatment methods could be applied to determine clopidol residue in chicken tissues.

### 3.03.P-Th158 Degradation, Leaching, and Ecotoxicological Behavior of Encapsulated and Nonencapsulated Biocides from Building Façades

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To preventively protect building materials from microbial infestation, the product formulations are provided with biocides. Environmental influences lead partly to UV-induced and hydrolytic degradation and transformation of the biocides already within the façade. Furthermore, rain events lead to the leaching of the biocides and their degradation products and thus to their release into the environment.

The aim of this study is to characterize the formation of biocide degradation products and to evaluate the effects on constitution and ecotoxicity of façade runoffs. Thus, render- and paint-based test façades were exposed to artificial weathering. Specimens containing the in-can preservatives MIT, CMIT, and BIT (nonencapsulated only) and the film preservatives TER, OIT and DCOIT (encapsulated or non-encapsulated) were irradiated in a weathering chamber using artificial sunlight and/or were treated according to the leaching test DIN EN 16105. Biocides and their degradation products were analyzed in the leachates and in the exposed façade matrix using HPLC-UV/VIS and HPLC-MS/MS. Ecotoxicological effects of the leachates were tested on luminescent bacteria and green algae.

For both irradiated and nonirradiated samples, it could be shown that nonencapsulated (ne) biocides are washed out to a higher extent than encapsulated (e) biocides (nonirradiated: TER (ne) up to 4 x higher, OIT (ne) up to 8 x higher; irradiated: TER (ne) up to 3 x higher, OIT (ne) up to 4 x higher). Consequently, samples with nonencapsulated biocides exhibit a higher toxicological behavior to green algae (nonirradiated:  $G_a$  (ne): 32,  $G_a$  (e): 16-24; irradiated:  $G_a$  (ne): 16-32,  $G_a$  (e): 12-16). Regardless of irradiation and encapsulation, all leaches showed a high toxicological effect to luminescent bacteria ( $G_l$ : 96). Furthermore, it could be shown that less biocides are leached from irradiated test façade, resulted by the UV-induced degradation of the biocides, which is in line with the increased detection of degradation products of nonencapsulated biocides in the leachate and in the façade matrix.

### 3.03.P-Th159 Release of Organic Contaminants from Geosynthetics in Dynamic Surface Leaching Test - Nontarget Screening, Target Analytic, and Effect-Based Identification

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Geosynthetics are often used for traffic route construction, where they are in contact with water and soil. In this study, we investigated geotextiles which were used for waterway construction. This material consists mainly of synthetic needle-punched polymer fibers (e.g., polypropylene or PET) which allows the material to be suitable for bank stabilization and filtration. Avivage agents are used in large quantities to optimize the manufacturing process and to improve the material properties (e.g., light and oxidation stability). The material is laid in stitched lanes along riverbanks and is covered with armoring stones or sand. The tensile strength and flexibility of the material ensures its durability, prevents erosion of the substructure material, and ensures its stability over several decades. For this purpose, two typical nonwoven geotextiles with different polymer compositions were characterized and investigated in a laboratory release study which focuses the dynamic surface leaching (DSL: DIN CEN/TS 16637-2:2014). The test consists of eight elution steps over a total period of 64d (days). The intervals for the water changes increases from initial step for each step, so that the first step lasts 6h and the following ones 18h, 1d 6h, 1d 18h, 5d, 7d, 20d, and 28d. Our study consists of the quantification of the total dissolved organic carbon (DOC), several target compounds as well a nontarget screening approach to identify relevant organic substances which were released from the material in the leachate. The number of components and the overall intensity decreases over the elution steps. The detected components were sorted to different levels of identification and prioritized compounds were quantified with target analytics. The identified substances can be assigned to the groups of sulfates, (benzene)sulfonates, polyethylene glycols (PEG), phosphates, and PET oligomers. One of the identified compounds, which leaches out of just one of the geomaterials, showed effects via bacteria luminescence inhibition tests and can be classified as ecotoxicologically relevant. The DOC content in the leachates of the geotextile from which the ecotoxicologically relevant substance leaches out is significantly lower than in the leachates of the geotextile which show no effects. As a result, (eco)toxicity cannot be predicted by the DOC alone, but needs to be addressed on the ecotoxicological effects measured.

### 3.03.P-Th160 Toxicology of Eluates from an Early Stage façade Lifetime and the Impact of UV Irradiation and Encapsulation

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Biocides are used as film and in-can preservatives are applied in paint and render formulations to protect building facades from microbial colonisation and biological decomposition. In-can preservatives leach out at the beginning of a façade lifetime, whereas film preservatives leach out over a long period in small concentrations. Consequently, these biocides were reported to be found in rivers and sewage systems.

The goal of this study was to chemically characterize façade runoffs obtained from free weathering experiments as well as from lab experiments after defined treatment and to evaluate the ecotoxicological effect of the resulting eluates on soil, sediment and water organisms.

Therefore paint/render systems were prepared as test samples being either biocide free, containing film and in-can preservatives or only containing in-can preservatives. The samples were collected after every rain event and immersion cycle, diluted with a dilution factor of 5.19 to reflect additional rain caused dilution and analyzed via HPLC-DAD or MS. The toxicity of the generated leachables were evaluated for several aquatic and sediment organisms, namely luminescent bacteria, green algae, umu-test, fish-egg test, *Chironomus riparius*, and *Lumbriculus variegatus*. The general leaching trend for both tests was the same, the free in-can preservatives with higher water solubility (especially BIT) leach out at the beginning of a façade lifetime in high concentrations and were not found after the first rain events / immersion cycles. Even considering high dilution, BIT shows toxic effect on various aquatic and sediment organisms. The more hydrophobic film preservatives, which were additionally encapsulated, leach out in low concentration over the complete period. In-can preservatives related effects could be observed for luminescent bacteria. Film preservatives mostly affect the toxicity for organisms like green algae. Even for more complex organisms like *Chironomus riparius* and *Lumbriculus variegatus* toxic effects for the natural weathering eluates could be observed. In the risk assessment of complex building materials, previous studies have mainly considered film preservatives, as those have a long service life and continuous leaching behavior. Therefore, despite their short-term toxic effects, these substances can be a stress factor for the environment and should not be neglected in the risk assessment of facades even if they degrade rapidly.

### 3.03.P-Th161 Removal and Biodegradation of Quaternary Ammonium Biocides in Wastewater Treatment

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Quaternary ammonium compounds (QUATs), are the most commonly sold biocides in Europe and several thousand tons are marketed every year for use in human and veterinary hygiene, wood protection, as well as other applications. However due to their surfactant nature it is not easy to determine basic physicochemical data and thus predictions for the behaviour in wastewater treatment are difficult to make.

Thus we studied mass balances and metabolism of QUATs in municipal wastewater treatment. Inflow and effluent concentrations were determined by means of HPLC-MS/MS from 24 h composite samples from three smaller WWTPs in Denmark. Especially Benzalkonium chlorides (BAC) and Dimethyl-Diakyl Ammonium chlorides (DDAC) were determined. Especially BAC-12, -14, and -16 as well as DDAC-12 were detected in all WWTPs. The highest concentrations in WWTP influents were measured for DDAC-12 (1000-4900 ng/L) followed by those for BAC-12 (900-2200 ng/L), BAC-16 (88-217 ng/L) and BAC-14 (25-100 ng/L). As the effluent concentrations were very small, the removal in the tested WWTPs was high (exceeding 99%). According to the sorption studies, this could completely be due to sorption, as sorption alone could result in 99% removal. However, also biodegradation contributes to the removal: for some compounds this is significant.

Biodegradation rate constants for a large variety of QUATs in sludge were determined in incubation experiments. The resulting half-lives ranging from 1.5-60 h. Concerning the congeners found in Danish WWTPs the spread was on average: 13 h (BAC-16) and 14 h (BAC-14), 31 h DDAC-12 and 49 h (BAC-12). Considering the relative rapid biodegradation of BAC-16, it can be assessed that biodegradation is also contributing the high removal of BAC-16. Thus there are two issues concerning the emissions of WWTPs: the parent compounds as sorbed to sludge pose a risk for terrestrial ecosystems. The formed metabolites are probably more water soluble than the parent compounds and might well be discharged with the effluent water to a relative high extent.

### 3.04.A Application of Biomonitoring Approaches to Support Surveillance of Chemical Exposure in the Environment

#### 3.04.A.T-01 Complementing Active Biomonitoring with Proteomics in an Aquatic Invertebrate Sentinel Species, *Gammarus Fossarum*

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Environmental risk assessment of chemical mixtures is challenging because of the multitude of possible combinations that may occur. Biological approaches, such as active biomonitoring to measure both bioavailable contamination and the biological responses of exposed organisms, may provide valid complementary tools to chemical monitoring. The freshwater amphipod *Gammarus fossarum* has been established to assess the contamination levels of a great variety of aquatic systems and the measurement of some specific molecular biomarkers is currently available in this species. In order to identify biomarkers or pathways of exposure and effect, it is necessary to screen and determine the most environmentally responsive molecular features

(e.g., proteins, metabolites). Multiple reaction monitoring (MRM) mass spectrometry is emerging as a relevant tool for measuring customized molecular markers in freshwater sentinel species. However, it is typically used for the validation of a limited number of molecular markers preselected from shotgun experiments at a whole-individual level in small invertebrates. This limits the physiological interpretation of these markers due to a lack of organ specificity. In this study, we used highly multiplexed MRM mass spectrometry to conduct large-scale screening of organ-specific proteomes in the sentinel species *Gammarus fossarum*. By combining laboratory and active biomonitoring (*in situ* caging) approaches, and MRM technologies, this study proposes a new strategy for the discovery of candidate molecular markers in the caeca, one of the main detoxification and metabolically active organs in amphipods. Seven hundred and sixty-five peptides representing 450 distinct proteins were monitored in MRM, resulting in a comprehensive coverage of biological functions. This MRM method was then successfully applied to analyse the caeca of gammarids either exposed in the laboratory to several environmental concentrations of cadmium (Cd), silver (Ag) and zinc (Zn) or caged in river sites across France with various types and levels of metal contamination.

Differential proteomics and functional analyses indicate that the proteome of *Gammarus fossarum* caeca show metal specific responses to different metal exposures, although the transfer of protein biomarkers to field biomonitoring still represents many challenges.

### 3.04.A.T-02 Temporal Suspect Screening of the Aquatic Invertebrate *G. pulex*

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There are over 350 000 chemicals licensed for manufacture and sale globally, including pharmaceuticals, pesticides, and personal care products. Through various routes, these chemicals are regularly detected in waterways. Where a chemical is detected in water, the biota that reside therein are exposed. To understand the effects on aquatic invertebrates, analytical methods must be developed to detect the contaminants that they are exposed to. However, the huge range of chemical pollutants means that developing targeted techniques to monitor the vast numbers of potential pollutants is a mammoth task. High-resolution mass spectrometry has made it possible to perform “suspect screening” – using a large library to tentatively identify analytes present in full-scan data. This is useful when it is impractical to obtain the number of reference standards required. Using the results of suspect screening means that the development of targeted techniques can be prioritised.

The aim of this study was to use suspect screening to monitor *G. pulex* to understand where prioritisation of techniques should be focussed.

*G. pulex* and river water samples were taken over the course of six months. Suspect screening was performed using a Shimadzu LC-QTOF-MS system, and a suspect screening was performed using a library of 1,195 compounds.

A total of 55 analytes were detected in *G. pulex*, predominantly pharmaceuticals, but also pesticides, illicit drugs, and metabolites. These results may be explained by the nearby wastewater treatment plant, which contributes to the high proportion of pharmaceutical pollution. Quantification was only possible for the antidepressant drug citalopram, whose concentration in *G. pulex* ranged from 52 to 107 ng/g. This concentration was plotted against the water temperature over the six month period and there was a moderate correlation, suggesting that there may be a connection between temperature and extent of contamination.

Although suspect screening has successfully been used in river water, using this method to screen aquatic invertebrates is novel and valuable because it highlights the contaminants that the biota absorb, which can be selected for further investigation to determine behavioural or biochemical changes. The results of this study also revealed an implication for a relationship between temperature and concentration of pharmaceuticals, which may be vital to understand an additional detail in the impact of climate change on chemical pollution.

### 3.04.A.T-03 First Evidence of Widespread Anticoagulant Rodenticide Exposure of the Eurasian Otter (*Lutra lutra*) in Germany

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Exposure of wildlife to anticoagulant rodenticides (ARs) has been extensively monitored and documented worldwide for a variety of terrestrial species that are directly or indirectly linked to pest rodents (the supposed target organisms and vectors of these biocides) via the terrestrial food web. Over time, more pieces have been added to the puzzle, unraveling the widespread AR contamination of other terrestrial nontarget organisms, including primarily exposed invertebrates and tertiary exposed predators. In recent years, the scientific focus of environmental AR monitoring extended to a former blind spot, namely AR emissions to the aquatic environment, and provided clear evidence of hepatic rodenticide residues in various freshwater fish species. As second-generation ARs are likely to be transferred along the aquatic food chain, this study investigated a total of 122 Eurasian otter (*Lutra lutra*) individuals found dead in Germany between 2005 and 2021 regarding hepatic residues of anticoagulants to shed more light on aquatic exposure pathways of ARs and involved environmental risks. Target analytes comprised one pharmaceutical and 8 biocidal anticoagulants as well as one suspected environmental metabolite. Notably, all target compounds

were detected in the analyzed livers of otters, a fish-eating top predator and sentinel species for the aquatic environment that is strictly protected by conservation laws. In regions with pronounced rodent control measures, otters were frequently exposed to biocidal ARs in spite of strict regulations regarding the sale, supply, and use of ARs. The elevated levels of second-generation ARs measured in several otters, compared to freshwater fish, confirmed their transfer along the aquatic food chain. Our monitoring data corroborate the predicted unacceptable risks of secondary poisoning for fish-eating mammals and birds during environmental risk assessment of second-generation ARs and their high potential for bioaccumulation and biomagnification. Thus, risk mitigation measures and restrictions of use that were implemented in Germany within the biocidal product authorization may not be protecting aquatic nontarget wildlife as intended. Improvements of stipulated measures are required to minimize the almost ubiquitous occurrence of hepatic second-generation AR residues in nontarget wildlife as the potential ecotoxicological consequences thereof are yet unknown.

#### **3.04.A.T-04 Using the Northern Gannet (*Morus bassanus*) to Monitor Environmental Changes of PFAS Prior and After Restrictions**

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Perfluoroalkyl substances (PFAS) are a large group of manmade compounds, produced since the 1950's, with chemical and thermal stability. Because of their persistence, ubiquity in the environment, bioaccumulation potential, and toxicity they are considered compounds of concern: PFOS (perfluorooctanesulfonic acid) and its related compounds are listed in the Stockholm Convention (2009).

We analysed long term trends of eight Perfluorinated Carboxylic Acids (PFCAs) and three perfluorinated sulfonates (PFSAs) in a marine sentinel species, the Northern gannet, in two UK colonies (Ailsa Craig, and Bass Rock) between 1977 and 2014.

We found no significant differences in the  $\Sigma$ PFAS between both colonies also that they were dominated by the  $\Sigma$ PFSAs which accounted for >80% of the total load measured in the eggs. By contrast, the  $\Sigma$ PFCAs was slightly, but significantly higher in Ailsa Craig (2-34.5 ng/g w.w.) than in Bass Rock (1.6- 26.4 ng/g w.w.). This group of compounds was dominated by perfluorotridecanoate (PFTrIDA), perfluoroundecanoate (PFUnA) and perfluorooctanoic acid (PFOA), with these compounds comprising 90% and 82% of the total in Ailsa Craig and Bass Rock, respectively. No significant difference in the  $\Sigma$ PFSAs (16.5 - 165 ng/g w.w.) were found between the colonies. PFOS dominated the PFSAs, accounting for 99% of the total concentration of sulfonates.

The  $\Sigma$ PFSAs and  $\Sigma$ PFCAs had very different temporal trends. The  $\Sigma$ PFSAs increased significantly in eggs of both colonies in the earlier part of the study. However, from the 1990s onwards there was a significant decline, possibly as a result of the phasing out of PFOS production in 2000. By contrast, in the 1970s and 1980s the concentrations of PFCAs were low and remained low, suggesting not much usage of these compounds. Then, their levels increased significantly in both colonies until the end of the study. The increase in  $\Sigma$ PFCAs was linked to the rise in large chain, namely odd, perfluorinated carboxylic acids. PFOA, the best known of the PFCAs, had a different temporal trend from the other predominant acids, showing a slow decline in the last 15 years of the study. In the first 20 years, PFSAs accounted for >90%  $\Sigma$ PFAS in eggs but from 1994 onwards, this proportion decreased to 62% - 73%, suggesting a temporal decline of PFSAs combined with increase of PFCAs.

#### **3.04.A.T-05 Legacy and Emerging PFAS in Cetacean and Seal Species Stranded Around the UK: Using UPLC-HRMS to Characterise Chemical Burdens Between Species**

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that are characterised by a hydrophilic moiety (e.g.,  $\text{SO}_3^-$  or  $\text{COO}^-$ ) and a hydrophobic perfluorocarbon chain that varies in length. PFAS display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence, and toxicity. However, only some of these substances, particularly the longer-chain PFAS, have been regulated by the Stockholm Convention and other international regulations. As a result, shorter-chain alternatives are being increasingly used in industry and, therefore, there is a growing need to document evidence of these emerging chemicals of concern in the environment. Liver samples can be used to determine the body burden of PFAS chemicals in marine mammals. Here, we employ an ultra-performance liquid chromatograph coupled to a high resolution mass spectrometer (Orbitrap) to analyse a range of both legacy and emerging PFAS including 11 PFCAs (C4-C14), 9 PFSAs (C4-C10), 3 FASAs, 3 PFPIAs and 6 fluorotelomer acids, among others. We have analysed the liver tissue of 130 individuals from a variety of species including bottlenose dolphins, humpback whales, and harbour seals stranded between 2012 and 2022. We report high total PFAS concentrations in a range of species of marine mammals collected around the UK. Concentrations of different compounds vary highly within species, but most species tend to be dominated by PFOS and long chain PFCAs. Contamination profiles reveal a potentially important route of exposure through the atmospheric transport of precursor compounds and a varying bodily response to *in vivo* PFOSA degradation between species. By quantifying and identifying PFAS in marine mammals, not only can we estimate bioaccumulation factors for specific chemicals, but we can also provide evidence for emerging chemicals that are not yet regulated. Our research addresses the need to

understand exposure levels in wildlife to PFAS compounds and also how PFAS fingerprints vary between different species over time.

### 3.04.B Application of Biomonitoring Approaches to Support Surveillance of Chemical Exposure in the Environment

#### 3.04.B.T-01 Characterization of Stream Communities Affected by Pesticides

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Pesticides biomonitoring is important to evaluate the effects of pesticides on nontarget organisms for sustainable management of aquatic ecosystems in agricultural landscapes. However, it remains a challenge to evaluate pesticide specific stress. This study gives a comprehensive characterization of two streams of the Swedish pesticide monitoring programme as well as a reference stream to elucidate pesticide-specific effects on the stream ecosystem. Besides the pesticide monitoring, i.e., weekly time-integrated surface water sampling and biomonitoring of benthic invertebrates and diatoms each fall, two sampling campaigns were conducted in early summer and fall 2022. Among other samples, benthic invertebrates and biofilm samples were collected for chemical analysis (110 pesticides and metabolites) and ecological analysis (species identification and fatty acid analysis).

The concentrations of 110 pesticides in benthic invertebrates, biofilms, water, and sediment were quantified and the benthic invertebrate and biofilm samples identified to species. Results between the two sampling seasons will be compared and put into relation with agricultural use data (i.e., amount of active substances sprayed in the catchment, crops grown, etc.). Taxonomy data and pesticide concentrations are used to calculate biological indices (SPECIES At Risk (SPEAR) index, TUmax, Average Score Per Taxon (ASPT), EPT). Distribution, occurrence, and correlations between measured concentrations in all matrices and biological indices will be evaluated with a follow up discussion about the expressive power of the indices to assess ecological effects of pesticides in agricultural streams.

#### 3.04.B.T-02 An Integrated Biological Effects Assessment of an Offshore Oil and Gas Installation in the North Sea

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The following study was part of the Norwegian Water Column Monitoring (WCM) programme, which performs investigations into the potential effects of offshore oil and gas activities on the marine environment. An integrated biological effects monitoring programme was designed to determine the potential effects of Ekofisk and Eldfisk oil and gas installations. Produced water (PW) impacts were assessed in the water column by deploying monitoring stations containing mussels, passive samplers (PSDs), and scientific equipment downstream of the Ekofisk and Eldfisk installations for 6 weeks at 2 depths between March and May 2023 and compared to two reference stations and a day zero group. Chemical and biological effects were measured and a clear relationship between PAH concentration in mussels and proximity to the Ekofisk installation was shown in the 20 m mussels reaching background concentrations in mussels 4000 m downstream. Only low concentrations were found in the Eldfisk mussels and 40 m mussels showing low exposure to the PW plume. Biological responses in relation to proximity to the Ekofisk installation were only found for some biological effects representing a weak response to low PW exposure. In addition, demersal fish were collected from within the Ekofisk safety zone and three regions of the North Sea (Ekofisk region, Egersundbank, Vikingbank). The fish species sampled included: cod (*Gadus morhua*), dab (*Limanda limanda*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangus*). Integrated chemical and biological effects were measured and a clear relationship was found between PAH exposure (liver /metabolites) in dab and CYP1A activity (EROD and CYP1A protein), with higher levels in dab populations living in areas of oil and gas activity. Genotoxicity was also observed in cod and dab from the Ekofisk, however, higher level orders of biological response such as histological changes were absent.

#### 3.04.B.T-03 MALDI-Mass Spectrometry Imaging Applied to Environmental Problems

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MALDI-Mass Spectrometry Imaging (MSI) is a multiomic technique that can record the mass spectrum of multiple analytes in a 2D matrix, usually sections of tissues or of a complete organism. MSI can be applied to locate the spatial distribution of biomolecules (e.g.: proteins, peptides, lipids, metabolites, etc.) in biological samples, and this information can be later combined with histological information from classical staining techniques to get molecular morphological maps. Here, we have used this approach to evaluate pollution effects in aquatic environments by analysing *Scrobicularia plana* clams. This bivalve mollusk is widely distributed in European intertidal mudflats and is considered a good bioindicator of exposure and bioavailability to metallic/organic contamination. A complete peptide and lipid maps from *S. plana* were generated, allowing the spatial definition of the different organs of the bivalve (i.e.: digestive gland, stomach, siphons, and foot) at a molecular level. Also, we have compared the peptidome and the lipidome of clams from different areas of the southwestern Andalusian coast and changes are being correlated with metal contents. The peptide analysis showed significant changes in animals from the Guadalquivir estuary,



specifically in their siphons. Furthermore, in the lipidomic analysis specific  $m/z$  values were associated to the different organs of the clam, and some of them presented altered patterns at different locations of the Huelva estuary. To our knowledge this work is the first molecular imaging mapping of clams and the first application of this technique for biomonitoring the effects of pollution in the environment.

#### **3.04.B.T-04 Plastic in the Air?! – Spider Webs as Spatial Mirrors for Microplastics and Tire Wear in Urban Air**

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Urban air is highly imprinted by anthropogenic activities resulting in micro-sized particulate matter release. Besides dust, soot, brake, and tire wear particle abrasion (TWP) the content of common microplastics (MP) in air, their composition, distribution, and transport potential have come into the focus of investigations recently. However, available quantitative data sets are highly limited.

Spider webs act as nonselective, climate-, and season-independent particle traps, which occur worldwide. They are costless and renewable. Successfully applied as passive samplers for inorganic contaminants 30 years ago, we investigate spider webs as a cheap and easily accessible biomonitor for evaluation of MP and TWP composition, spatial distribution, and relative concentrations in urban air.

Samples were collected in covered bus stops at different trajectories and times in a mid-sized German city. The spider webs were processed with an advanced oxidation process (Fentons reagent) and analysed with PY-GC/MS. Polymers were quantified as backbone-related clusters (indicated by prefix “C”) as introduced earlier. Various soot samples were analyzed to evaluate potential interferences with the C-PVC indicator naphthalene; subsequently a factor was introduced to correct the C-PVC data (indicated by \*). TWP and “traditional” MP were present in all samples representing 1 to 10% of the web’s total mass. Most dominant was car tire wear (Ø 41%), followed by C-PET (Ø 36%) and \*C-PVC (Ø 12%). C-PET is most likely derived from textile fibers. \*C-PVC is assumed to originate notably from road markings. Although frequently found, truck tire wear, C-PE, C-PP, C-PS, C-PMMA, and C-PC were present in much lower concentrations (Ø <6.4%). TWP prevails in highly traffic-influenced sampling areas, while “traditional” MP dominated in residential areas. Spider webs are suggested as a globally applicable “pollution mirror” to get an overview of spatial (horizontal/vertical) as well as temporal MP contamination trends and to identify hotspots in (urban) air (outdoor/indoor).

#### **3.04.B.T-05 Identification of Unmonitored Aryl Hydrocarbon Receptor Agonists in Marine Biological Samples in South Korea Using Advanced Effect-Directed Analysis**

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In the present study, we investigated major aryl hydrocarbon receptor (AhR) agonists in marine biota samples using effect-directed analysis (EDA) combined with full-scan screening analysis (FSA). The specific objectives were to: i) investigate major AhR-active compounds in organic extracts of eggs and livers of black-tailed gulls and blubber, liver, and muscle of common dolphins and a fin whale using H4IIE-*luc* bioassay, ii) measure concentrations of known AhR-active compounds, including traditional and emerging polycyclic aromatic hydrocarbons (PAHs) and polar AhR agonists in biota extracts using GC-MSD and HPLC-MSMS, respectively, iii) identify tentative AhR agonists in highly potent fractions using high-resolution mass spectrometry, and finally iv) evaluate relative contributions of each AhR agonist to overall potencies. Significant AhR-mediated activities were observed in the mid-polar fraction of egg extracts of black-tailed gulls and the polar fraction of liver extracts of black-tailed gulls and blubber and liver extracts of fin whale. Known AhR agonists had limitations in explaining the total biological effects. FSA was conducted on mid-polar fractions of egg extracts using GC-QTOFMS and on polar fractions of liver extracts of black-tailed gulls and blubber and liver extracts of a fin whale using LC-QTOFMS. AhR agonist candidates were selected through the application of selection criteria. Of these, ethylbenzoate ( $6.0 \times 10^{-5}$ ), 3-phenylpropanal ( $2.0 \times 10^{-4}$ ), and 1,4-dicyclohexylbenzene ( $7.0 \times 10^{-4}$ ) of egg extracts were identified as novel mid-polar AhR agonists. Polar AhR agonists such as [10]-gingerol ( $4.0 \times 10^{-3}$ ), angelicin ( $3.0 \times 10^{-3}$ ), corticosterone ( $2.0 \times 10^{-2}$ ), eupatilin ( $3.0 \times 10^{-3}$ ), etofenprox ( $2.0 \times 10^{-3}$ ), oxadixyl ( $5.0 \times 10^{-4}$ ), tretinoin ( $5.0 \times 10^{-4}$ ) in liver extracts of black-tailed gulls, and raloxifene ( $2.0 \times 10^{-3}$ ) and fluphenazine ( $3.0 \times 10^{-4}$ ) in blubber extracts, peimisine ( $6.0 \times 10^{-3}$ ) and alantolactone ( $3.0 \times 10^{-3}$ ) in liver extracts, and hydroxygenkwanin ( $2.0 \times 10^{-4}$ ) in blubber and liver extracts of a fin whale were newly identified. With the addition of novel AhR agonists, the contribution increased by 0.001–86% in egg extracts, 27–52% in liver extracts of black-tailed gulls, 2.6%–16% in blubber extracts of common dolphins and a fin whale, and 49% in liver extracts of a fin whale. Further studies are needed to evaluate the quantitative ecological risk that can be expressed by the AhR-mediated activities in biological samples.

#### **3.04.P Application of Biomonitoring Approaches to Support Surveillance of Chemical Exposure in the Environment**

### **3.04.P-Mo157 Complementing the Assessment of the Chemical Status of Transitional and Coastal Water Bodies, Considering Both Waters and Biota**

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The Water Framework Directive, through its Directive 2013/39/EU, establishes how to carry out the assessment of the chemical status of water bodies, defining the quality standards for priority and preferential substances. The directive encourages the study of pollutants in water, irrespective of water type: river, groundwater, reservoir, transitional, or coastal water. In certain types of water or pollutant, this could be adequate, but when the variability of the water body is large, the characterisation of water pollutants becomes more complicated. Thus, the directive recommends periodic point sampling of such water bodies.

Biota is considered as an integrative, cumulative system over time, which, thanks to toxicity studies that validate the quality standards established for it, could indicate the degree of contamination of an area.

This directive establishes fish as the biota to be considered, except in the case of polycyclic aromatic hydrocarbons (PAHs), for which crustaceans or mollusks are indicated for monitoring.

In water bodies such as transitional water bodies interacting with the coastal zone and coastal water bodies, the assessment of chemical status with fish could be somewhat complicated, as fish do not distinguish humans established boundaries between water bodies.

Given that the variability of water in point sampling is very high, sediment and biota (i.e., fish), due to their integrative characteristic, could be considered as more suitable matrices for the assessment of the chemical status of transitional and coastal water bodies. Sediment could be used to study the temporal evolution of the status of a water body within the WFD. However, considering the mobility of fish, it is difficult to use them to evaluate, with any degree of confidence, the status for each water body.

Therefore, the Basque Water Agency (URA) takes into account punctual water samples throughout the year and molluscs in estuaries to monitor the chemical status in the Basque coast. Mollusc data is used to confirm the evaluation of those substances for which the level of confidence of the data in water is not high enough to make the evaluation, as is the case of PAHs, specifically Benzo(a)pyrene.

### **3.04.P-Mo158 Making Use of Long-Term Monitoring Data Sets: Factors Affecting Biomarkers Commonly Used in Environmental Monitoring Programmes**

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Anthropogenic chemical pollutants in the marine environment have the potential to accumulate in the tissues of organisms leading to biological disruption and disease which can adversely affect ecosystems. Long-term monitoring programmes like the UK Clean Seas Environmental Monitoring Programme (CSEMP) rely on biological effects techniques to assess if such chemicals are affecting sentinel species. We have applied mixed and fixed effect linear models to the long-term CSEMP dataset to evaluate if factors such as region (location), sex, age, condition factor (CF), and gonadosomatic index (GSI) contribute to the variability observed in the levels of 3 well established biomarkers (inhibition of acetylcholinesterase in muscle-AChE, induction of hepatic ethoxyresorufin-O-deethylase-EROD, and presence of biliary 1-hydroxypyrene-1-OH pyrene) used to monitor biological effects of contaminants in dab (*Limanda limanda*) in England. Regional differences (location) were a significant explanatory variable for the 3 biomarkers. We report for the first time that, while accounting for region, sex and gonadosomatic index are significant predictors of muscle Acetylcholinesterase isoform in dab. Dab condition factor is also a significant predictor for both enzymatic markers (AChE and EROD) but not for 1-OH pyrene. We observed a significant age and sex interaction for hepatic EROD, with male dab, above 2 years, having higher values when compared to females. While the results from this study confirm the adequacy of the current CSEMP biomarkers protocol they also highlight the factors to account for when analysing/assessing if environmental contamination is contributing to the responses of these 3 biomarkers in dab around UK waters.

### **3.04.P-Mo159 Whole Slide Imaging, Digitalization, and Automation of Histopathological Lesion Evaluation in Marine Organisms for the Purpose of Environmental Monitoring**

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Use of histopathology to identify changes in marine species in response to contaminants is a long-known practice. The suitability of histopathological biological markers in the assessment of marine environment quality is derived from its high ecological relevance, as alterations on the tissue level are often irreversible. Also, affecting the physiology of an individual could impose a potential effect on the whole population. Visual assessment, traditionally used in the scoring of histological parameters, is a tedious procedure, which requires highly trained personnel and it is often prone to bias. The advances in image acquisition and digital image analysis over the last decades has created a suitable, dynamically evolving environment for analysis of high-content histological images.

Herein, the use of digital pathology, taking advantage of computer vision and the high potential for automation, was utilized to analyse various tissues of both mussels (*Mytilus edulis*) and fish collected in the vicinity of an offshore oil and gas exploration platform in the North Sea.

The application of digital image analysis in performing analysis of histological parameters was implemented in the evaluation of gonads. Using QuPath, an open-source digital analysis software, specifically designed to handle whole slide images, we developed a method for gender recognition in Hematoxylin & Eosin (H&E) colored slides with >97% success rate. We performed a comprehensive analysis of other lesions and are working on developing scripts to analyse a selected set of lesions particularly relevant for the purpose of environmental monitoring. Obtained results were compared with the available scores from the manual assessment, in addition QAQC aspects (using independent and blind experts) were carried out to secure high quality data. Data regarding the liver tissue, center of the metabolic activity of the organisms are also presented.

### 3.04.P-Mo160 NORMAN Workshop: Improving the Use of (Semi-)Field Data for the Risk Assessment of Chemicals

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As part of the NORMAN network (<https://www.norman-network.net/>) Working Group 2: Bioassays, the Expert Group (EG) on “Ecosystem level effects of chemicals of emerging concern (CECs) on aquatic ecosystems” organised the workshop: ‘Improving the use of (semi-)field data for the risk assessment of chemicals’ on the 21<sup>st</sup> and 22<sup>nd</sup> of November, 2023. The aim of the workshop was to create a framework for the field-based calibration of the different (new) tools that can be used to assess the effects of chemical mixtures on ecosystems, with focus on the integration of higher tiers of biological complexity/organization. We propose a framework that integrates (i) analytical data of individual chemicals, (ii) effect-based monitoring data obtained by *in vitro* and *in vivo* bioassays with multiple endpoints accompanied by chemical measurements, (iii) PNEC values based on laboratory toxicity data for standard test species (i.e., from the NORMAN ecotox database) to assess risks of single substances, (iv) multisubstance potentially affected fraction (msPAF) calculations to predict the toxic pressure of mixtures, (v) mesocosm effect data (e.g., NOECs) into predictive mixture assessment tools like the PERPEST model, and (vi) biomonitoring data based on ecological (e.g., diversity indices) and biological effects assessment (e.g., biochemical, physiological and molecular markers) to evaluate mixture effects of contaminants in the environment, and explored how these can be integrated or compared for validation/calibration. As a proof of concept, we used a lowland stream monitoring dataset collected in Germany, which covered the majority of these data types (<https://doi.org/10.1594/PANGAEA.931673>) and focussed on pesticides. We added PNECs from the NORMAN ecotox database, msPAF values and effect data from mesocosm studies to this dataset to enable a linkage and comparison. A specific proof-of-concept study has been created which demonstrates how the validation and calibration of the different parts can be performed, and/or where the main uncertainties and data gaps are. The results of the workshop and regulatory implications will be discussed further and summarised for the SETAC meeting aiming to establish an applied framework for the integration of all these available resources for risk assessment.

### 3.04.P-Mo161 Contamination of Marine Biota and MSFD Environmental Status

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Under the European Marine Strategy Framework Directive, spatiotemporal trends of contaminants should be assessed on the entire exclusive economic zone. In the present study, the approach implemented to monitor and assess the chemical contamination of marine ecosystems in France, is described. Criteria have been identified i) to select sentinel species with different habitats, trophic levels/diets, and covering different dimensions of the marine environment, and ii) to limit the effect of confounding factors on bioaccumulation of contaminants. Then, two assessment approaches were employed; absolute assessments based on distance of contamination indices to thresholds, and relative assessments based on temporal trends. Among chemical contaminants, mercury (Hg) is used in the present communication as a “model” contaminant to illustrate our approach.

In bivalves from 36 coastal stations, Hg concentration indices ranged from 0.013 to 0.129 µg/g wet weight (ww). On the continental shelf, Hg indices ranged from 0.023 to 0.677 µg/g ww in fish (7 species), from 0.038 to 0.686 µg/g ww in birds (6 species) and from 6.8 to 15.4 µg/g ww in mammals (2 species). Increase in Hg concentrations with trophic position (bivalves < fish < birds < mammals) illustrates its biomagnification in trophic food webs. Absolute assessments showed that Hg concentrations were 0.7 to 6.5 times higher than their thresholds (0.02 µg/g ww) in bivalves and 1.1 to 33.9 times higher in fish, depending on the species and region. Though concentrations in birds and mammals were higher than in bivalves and fish, they were 0.02 to 0.69 times the selected thresholds (1 and 61 µg/g ww, respectively), depending on the species and region. This emphasizes that threshold selection and use are not trivial as absolute assessments depend on it. In bivalves and mammals, the two groups of species with sufficient temporal coverage for relative assessments, mercury concentrations increased over the last 20 years. The source of this increase is still to be characterized (e.g., increase in global emission, change in diet), but it indicates that individuals should continuously allocate energy for detoxification and adapt to this pressure (cost for adaptation). Overall, Hg is giving rise to pollution effect in French Atlantic marine ecosystems according to several lines of evidence. The present approach is ambitious and enables to assess environmental quality based on a comprehensive observation of the marine ecosystems.

### 3.04.P-Mo162 Assessment of Polycyclic Aromatic Hydrocarbons and Phenolic Endocrine-Disrupting Compounds in Seawater, Sediment, and *Posidonia oceanica* in Giglio Island (Arcipelago Toscano National Park, Italy)

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The Mediterranean coastal areas are characterised by different anthropogenic activities including tourism, shipping, and industrial activities, which are responsible for chemical contaminations. Unfortunately, there are evidences that also marine protected areas are not free from contamination. Many contaminants have mutagenic and/or carcinogenic properties, representing a risk for ecosystem health. The evaluation of chemical contamination in both biotic and abiotic matrices in environmental monitoring programs makes it possible to better assess chemical risk for ecosystems than those solely based on abiotic compartments. In this context, the Mediterranean endemic seagrass *Posidonia oceanica* (L.) Delile 1813 is currently used in several monitoring programs associated to metal contamination, but it has been recently proposed as a suitable bioindicator of organic contamination, due to its potential bioaccumulation capacity. Among contaminants, polycyclic aromatic hydrocarbons (PAHs) and phenolic endocrine-disrupting compounds (PEDCs), including bisphenol A (BPA), 4-nonylphenol (4-NP), and its ethoxylated precursors, have gained considerable attention for their potential toxic effects on humans and wildlife. PAHs are priority pollutants, to be monitored as tracers of anthropogenic impacts. BPA has been recognized as a 'possible future priority substance' in the Water Framework Directive and its use has been banned from infant plastic feeding bottles, toys, varnishes, coatings, and plastic food contact materials. 4-NP has been included in different EU regulations and in the list of priority hazardous substances to be eliminated in surface waters. In this context, the present study aims to evaluate the occurrence of PAHs and PEDCs in seawater, sediment, and *P. oceanica* samples collected at 4 stations located at increasing distance from the sewage pipeline of the small touristic town of Giglio Porto (Tyrrhenian Sea, Italy). The abundance of the autochthonous microbial communities in seawater and sediments, as well as in *P. oceanica* leaves and rhizomes was also investigated. Samplings were performed in early spring and late summer 2021, during the minimum and maximum level of tourist presence on the island, respectively. Target contaminants were detected in all matrices investigated suggesting their ubiquitous presence in this marine ecosystem. As expected, higher average concentrations of PAHs and PEDCs were observed in summer.

### 3.04.P-Mo163 The First Assessment of Halogenated Organic Compounds in the Blubber of Short-Finned Pilot Whales (*Globicephala macrorhynchus*) Stranded Along the Coast of Savu Island, Indonesia

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Short-finned pilot whales (SFPW) are frequently stranded cetaceans, although the cause of their stranding is unknown. Toxic, persistent, bioaccumulative, and endocrine disruptor substances, such as polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs), may threaten these cetaceans' existence. In this study, the contamination status, congener profiles, and ecological risks of PCBs, PBDEs, and other brominated flame retardants (BFRs), including decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), pentabromoethylbenzene (PBEB), and 2,2,4,4,5,5-hexabromobiphenyl (BB-153), were investigated. Blubber samples were analyzed from 20 of the 46 dead-stranded short-finned pilot whales (SFPW) on Haingahu Beach, Savu Island, Indonesia, in October 2012. PCBs and BFRs were extracted using a high-performance dispersion homogenizer and purified using a multilayer silica gel column (44% H<sub>2</sub>SO<sub>4</sub>-silica, 22% H<sub>2</sub>SO<sub>4</sub>-silica, and anhydrous Na<sub>2</sub>SO<sub>4</sub>) and activated silica column. PCBs and BFRs were quantified using GC-HRMS (EI-SIM) and GC-qMS (NCI-SIM). Our results show

that concentrations of  $\Sigma 209$ PCBs,  $\Sigma 7$ in-PCBs,  $\Sigma 12$ dl-PCBs, and  $\Sigma 21$ u-PCBs were between 48–490 (mean:  $240 \pm 140$ ), 22–230 ( $110 \pm 60$ ), 2.6–38 ( $17 \pm 10$ ), and 1.0–13 ( $6.3 \pm 3.7$ ) ng g<sup>-1</sup> lipid weight (lw), respectively. As for BFRs, concentrations of  $\Sigma 41$ PBDEs, PBEB, BB-153, and BTBPE were between 3.7–32 ( $13 \pm 8$ ), <0.01–1.8 ( $0.73 \pm 0.49$ ), <0.04–0.22 ( $0.16 \pm 0.045$ ), and 0.14–2.2 ( $1.3 \pm 0.50$ ) ng g<sup>-1</sup> lw, respectively. Although not significant, concentrations of  $\Sigma 209$ PCBs and  $\Sigma 41$ PBDEs in juveniles were relatively higher than those in subadult females and males. Congener-specific profiles of PCBs among sex and estimated age groups were observed; sub-adult females accumulated high proportions of hepta- to deca-CBs and recalcitrant structure-activity groups congeners compared to sub-adult male and juvenile groups. The estimated toxic equivalency (TEQs) value for dl-PCBs ranged from 2.2 to 60 TEQWHO pg g<sup>-1</sup> lw, with juveniles containing high TEQ values than sub-adults and adults. For BFRs, the most abundant congener detected was BDE 47, with a proportion of approximately 47%. Although the concentrations of PCBs and BFRs in SFPW stranded along the Indonesian coasts were lower than those reported for similar whale species from other North Pacific regions, further research is needed to assess the long-term impact of halogenated organic pollutants on their survival and health.

### 3.04.P-Mo164 A Biological Effects Assessment Tool: An Integrated Approach to Evaluate Mixture Effects of Contaminants on the Baltic Sea Biota

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Biological effect monitoring detects and assesses early biochemical or physiological changes caused by exposure to contaminants, most commonly in complex mixtures and under various environmental factors. To achieve Good Environmental Status (GES), the regulatory efforts, including Marine Strategy Framework Directive (MSFD), rely on ecological (based on the community status) and chemical (based on the contaminant levels) assessments. However, the essential link between those assessments is the ecotoxicological effect assessment of contaminants, which is currently missing. In this context, the challenge is in integrating monitoring data on the effects of contaminants across the different levels of biological organisation and species into a functional framework, which could then be linked to the contaminant levels in the environment.

Biological effects in biota exposed to diverse contaminant mixtures are often related to different mechanisms and modes of action. We propose a new approach for integrating these effects by defining operational sets of physiological traits, bioindicator species, and biomarkers that respond in concert. The strength of this integration could be used to detect and quantify responses to the entire array of stressors present in the study area. As a tool for assessing the co-variation across the response components, we will use multivariate ordination and geometrical properties (i.e., sphericity) of the response profile in the multidimensional space. The rationale is that the scatter of multivariate data consisting of various responses (i.e., biomarkers, physiological and population-level responses) measured in biological effect monitoring would exhibit a directional organisation appearing as an ellipsoid shape of the data cloud when multiple mechanisms are in action to overcome stress. By contrast, a circular or random pattern would suggest a non-stressful and healthy environment indicative of GES.

We will develop and explore this approach, including appropriate statistical properties, using data from the biological effect monitoring and studies applying various ecotoxicological methods to assess the ecological consequences of chemical pollution in the Baltic Sea. The work will be conducted as a part of the INTERREG Baltic Sea Region BEACON project aiming to develop environmental assessment tools. The outcome of the project will be instrumental for MSFD implementation by assessing the impacts of pollution in the Baltic Sea.

### 3.04.P-Mo165 Sharks as Biomonitorers of Plastic Pollution in Benthic and Pelagic Marine Ecosystems

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Plastic waste has increased markedly in the last decades and the aggravated effect of this super-production and inefficient waste management is that millions of untreated plastic tones end up in the ocean. The different types of plastics can have distinct behaviours on marine environments, sinking and accumulating in marine sediments or remaining in the water column, potentially affecting both benthonic and pelagic ecosystems. Long-lived top predators are prone to accumulate large quantities of plastic debris due to bioaccumulation and biomagnification processes. For that reason, and given the diversity of habitats they can occupy (i.e., from benthic to pelagic and from coastal to oceanic environments), carnivorous sharks can be considered good sentinels of microplastic contamination. The main objective of this study was to assess microplastic incidence in two carnivorous shark species inhabiting different environments within the Northeast Atlantic Ocean: the benthic small-spotted catshark (*Scyliorhinus canicula*) and the pelagic blue shark (*Prionace glauca*). Sharks were opportunistically captured aboard commercial fishing vessels operating in the North Atlantic (37 *P. glauca* and 44 *S. canicula*) and their stomachs stored for the analyses of microplastic ingestion. Stomach contents (*P. glauca*) and the entire stomachs (*S. canicula*) were digested with 10% potassium hydroxide (KOH) at room temperature, added in a 1:3 proportion (m:v), and filtrated in order to identify, count, and characterize microplastic particles. The results showed a prevalence of 100% in the frequency of occurrence of microplastics in the stomachs of the sampled sharks. Notwithstanding, the abundance and type of plastics present in each species was substantially different. The benthic *S. canicula* accumulated mostly microfibers (88% fibers, 9% fragments, and 3% filaments) but in relatively low numbers (around 7-8 microplastics per individual), while the pelagic *P. glauca* accumulated uncountable numbers of

microfragments (i.e., hundreds). These results are in accordance with previous studies of microplastic contamination in marine ecosystems, being fibres more associated with benthic environments, and fragments with pelagic individuals. Our study highlights the high susceptibility of larger sharks to the contamination by microplastics, raising concerns on their potential negative effects.

### **3.04.P-Mo166 Monitoring the Co-Occurrence of Microplastics and Chemical Contaminants in Freshwater Invasive Mussels**

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Because bivalves are sessile, relatively tolerant to toxic compounds, and abundant in aquatic ecosystems globally, they are often used to monitor chemical contaminants. Since 1992, NOAA's Great Lakes Mussel Watch Program has used invasive zebra and quagga mussels (*Dreissena* spp.) as biomonitors for chemical contaminants. Microplastic concentrations in the Great Lakes are similar and sometimes higher than in the ocean, suggesting dreissenid exposure. Although dreissenids ingest microplastics in the lab, no studies have examined spatial and temporal patterns of microplastics *in situ* for Great Lakes dreissenids. We compared microplastics in dreissenids among sites, mussel size classes, and sampling dates and examined the relationship between microplastics and contaminants, while considering if these mussels could be valuable for monitoring both contaminants and microplastics in the Great Lakes. Mussels were harvested from reference sites and sites influenced by wastewater effluent and urban river discharge in Milwaukee Harbor (Lake Michigan, USA). Caged mussels were deployed in summer for 30 and 60 days, sorted by size, and analyzed for microplastics and body burden of three contaminant classes: alkylphenols, polyaromatic hydrocarbons, and petroleum biomarkers. The number of microplastics in dreissenid mussels was similar to published values for other bivalve species globally. Microplastics in dreissenids were highly variable with no clear link to pathways of introduction (wastewater discharge, river confluence) or a consistent site trend. At the wastewater site, microplastics were higher in the largest mussels after 30 days. However, for smaller mussels, no distinction among sites was apparent with no differences among sites after 60 days. Out of 93 contaminant comparisons, the only positive correlation was between microplastics and three PAHs (decalin, methylnaphthalene, 1-methylfluorene). Our initial conclusion is that dreissenid mussels in the Great Lakes are not reliable biomonitors for microplastics because variation in microplastics among individuals, size classes, and time periods was higher or equal to the variation among sites. It is clear that intrinsic and extrinsic factors influencing mussel-microplastic interactions require further examination. Even so, our data contribute to the understanding of microplastic spatial distributions in urban freshwaters, the role of dreissenid mussels in plastic budgets, and models for the fate of microplastics.

### **3.04.P-Mo167 Egg Yolk and Albumen as Biomonitoring Tissues for Organic Pollution in Maternal Foraging Grounds**

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The composition and concentration of chemical cocktails are often poorly established in marine environments particularly beyond the coastal zone. Marine migratory species could act as biomonitors to represent the exposure of associated communities, provided that suitable tissues for biomonitoring are available. Sea turtles could present suitable biomonitors because they integrate pollution across their long lifespan and wide migrations. They also do not feed during their nesting migrations, making their eggs potentially suitable biomonitoring tissues to reflect pollution in maternal foraging grounds. We investigated maternal transfer among 56 PCB, 12 OCP, and 34 PBDE compounds from coupled samples of loggerhead turtle maternal plasma and replicate egg yolk and albumen. We applied robust Regression on Order Statistics to calculate summary statistics among samples with measured concentrations below detection limit. Wet weight concentrations of compounds were lower and more variable among replicate samples in albumen than yolk. Additionally, the correlation with plasma concentrations was lower for albumen than yolk, which discourages the use of albumen as a model tissue for maternal burdens at the current moment. By contrast, lipid-normalized concentrations of PCB and OCP were positively correlated between plasma and yolk, and overlapped with previous observations between whole blood and whole egg for leatherback and green turtles. The maternal transfer rate between yolk and plasma indicated that lipid normalized concentrations in yolk were on average 6.6 times lower than those in plasma. Nevertheless, this maternal transfer rate varied between compounds suggesting compound-specific transfer, and negatively related with compound lipophilicity as estimated by the octanol-water partitioning coefficient,  $K_{ow}$ . Dioxin-like PCB 118 was among the three PCB with highest concentrations in yolk, and obtained a high  $\log_{10}$  maternal transfer ratio of -0.30. Regarding OCP, p,p'-DDE was the most prevalent compounds in yolk, albumen, and plasma, and obtained a near-equilibrium  $\log_{10}$  maternal transfer ratio of 0.01. Our results indicate that yolk could offer a suitable biomonitoring tissue which relates to maternal body burdens. Nevertheless, compound-specific maternal transfer rates need to be considered when translating yolk levels back to maternal pollution burdens, and when assessing the risk to the subsequent generations of turtle embryos.

### **3.04.P-Mo168 Seasonal Variation in Size, Stable Isotopes (Carbon and Nitrogen), and Fatty Acids in Riparian Sentient Spiders (Tetragnathidae)**

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Riparian spiders are used in ecotoxicology as bioindicators of aquatic to terrestrial transfer of energy and bioaccumulative contaminants through the insect mediated contaminant pathway. Spiders in the family *Tetragnathidae* are particularly of interest because of the high amount of emergent aquatic insects in their diet. These spiders have become more well studied in recent years; however, the changes in their diet and size throughout a year have not been investigated. In this study, our objective was to determine if variation in size, carbon and nitrogen stable isotopes, and the fatty acid biomarker eicosapentaenoic acid (EPA) occurs in tetragnathid spiders throughout a year. Spiders were sampled within a 100m reach of the East Fork Stones River in

Cannon County, TN twice a month between April and November 2021. It was found that spider total mass and body measurements steadily increased from April to September, then decreased in October. It was also found that carbon and nitrogen stable isotopes were consistent over time and that the %EPA of total fatty acids in spiders varied greatly, with spikes in May and August. This work adds to the growing literature on the use of tetragnathid spiders as bioindicators. The implications of this research show that seasonality may not be a significant factor when considering stable isotope or EPA as food web tracers.

#### **3.04.P-Mo169 Biomonitoring of Maritime Traffic and Tourism Recovery Post-COVID-19 lockdown in the MPA of Ischia Island (Italy) Using *Mytilus galloprovincialis***

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Ischia is the biggest island in the Gulf of Naples, and constitutes the most important part of the Regno di Nettuno's marine protected area (MPA). This island is historically characterized by a high number of tourists, a fact that leads to a high anthropic impact on coastal seawaters due to intense maritime traffic and mass tourism. During the first Sars-CoV-19 pandemic lockdown that occurred between March and June 2020, the island was almost completely isolated by the land for 3 months. This event represented a unique opportunity to understand the temporal and quantitative trend of the release of human-discharged chemicals in seawater and their potentially deleterious effects on coastal seawater biota. Therefore, an active biomonitoring was carried out transplanting adult mussels of the species *Mytilus galloprovincialis* in three coastal sites of the island (Ischia Harbour, San Pietro shore, and Castello Aragonese). The accumulation in soft tissues of chemicals that are suitable marker of domestic waste such as caffeine, carbamazepine, and N,N-diethyl-meta-toluamide was measured through UPLC/MS. A suite of biomarkers related to energetic metabolism, detoxification, oxidative stress, and oxidative damage was also applied. The results showed that pollutants related to day-life activities are significantly released at sea and bioavailable for filter feeders and can represent an actual risk for local benthic communities. Indeed, the metabolic functions and biochemical performances resulted significantly modulated in translocated organisms and remarkable level of lipid peroxidation was observed. The application of a biomarkers Response Index allowed distinguishing impacts of pollution, showing mussels from Castello Aragonese as the most threatened. The approach adopted in this study emerged as a useful tool to point out the potential vulnerability of coastal areas with high natural values, contributing to their management and conservation.

#### **3.04.P-Mo170 Monitoring of Contaminants in the Northern Baltic Sea: Toward an Integrated Approach**

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The Baltic Sea is heavily polluted by a large variety of chemicals. While the relatively small number of "legacy" contaminants have been addressed in the monitoring programs, there is a wide array of emerging substances present in the marine environment that bioaccumulate and cause harmful effects in organisms. Therefore, there is an urgent need for the development and implementation of modern integrated monitoring approaches.

Biomarkers can be used as early warning signals to measure stress caused by environmental conditions, including contaminants, to biota. In the Finnish monitoring programme, lysosomal membrane stability (LMS) has been regularly analysed in perch (*Perca fluviatilis*) and herring (*Clupea harengus membras*) since 2014 from coastal and open sea areas. The caging of mussels (*Mytilus trossulus*) was used to study pollution at different coastal sites by analysing tissue concentrations of selected contaminants in combination with various biological effects methods including biomarkers of oxidative stress, neurotoxicity, biotransformation, and the condition index. In addition, polydimethyl siloxane (PDMS)-passive samplers attached in the cages detected the presence of PAHs, PCBs and organotins in water. To gain a deeper understanding of the chemical compounds present in these areas, target and nontarget screening methods were applied in mussel and fish samples. The targeted analysis included more than 2000 different chemicals and the nontarget screening around 90 000 substances. The sampling sites included both heavily polluted sites (e.g., near a wastewater treatment plant outlet) and areas away from point pollution sources.

The results showed significant differences in the accumulation of contaminants and the measured biological effects between the study sites. Moreover, different groups of contaminants were detected in different species; e.g., PFASs and PCBs were commonly detected in fish, but less in mussels while PAHs and organotins were typically detected only in mussels. Passive samplers mimic accumulation of chemicals to biota and passive sampling has proven to be an efficient technique to study, e.g. organotin compounds in aquatic environment as also shown in the present study. Using different chemical-biological methods in the environmental monitoring enables to estimate the exposure and effects of emerging contaminants in the aquatic environments to gain a more holistic view of the contamination status of the Baltic Sea.

#### **3.04.P-Mo172 Flame Retardants in Eggs of Black-Tailed Gull from its Breeding Sites Along the Korean Coast**

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Seabird has long been used as a biomonitor for long-term monitoring of environmental contaminants in the Europe and North America. This study was performed to identify the levels and profiles of flame retardants (FRs), emerging persistent organic pollutants (POPs), in seabird eggs inhabiting along the Korean coasts. Black-tailed gull eggs were collected from breeding places located in the southern (Hong-do), eastern (Dok-do), and western (Seoman-do) coasts from 2015 to 2020, and egg content was used for chemical analysis. FRs were widely detected in seagull eggs. The overall concentration of FRs in the egg were in the range of 19-1280 (median value: 74) ng/g lipid wt. for polybrominated diphenyl ethers (PBDEs), 28-1141 (136) ng/g lipid wt. for hexabromocyclododecanes (HBCDs), and 0.8-131 (11) ng/g lipid wt. for novel brominated flame retardants (NBFRs) including PBT, PBEB, DPTE, HBBz, HCDBCD, BTBPE, OBIND, and DBDPE. Among the target analytes, HBCDs showed the highest concentration and followed by PBDEs and NBFRs. NBFRs were detected at concentrations 5-19 times lower than PBDEs and 8-14 times lower than HBCDs. The levels of PBDEs and HBCDs were relatively high in the western coast (Seoman-do), while NBFRs showed no significant regional differences. No differences in NBFRs were observed while an increasing and decreasing trends for PBDEs and HBCDs were observed during 2015 to 2020.

### 3.04.P-Mo173 Determination of Atmospheric Microplastics in Moss with TED-GC-MS and $\mu$ Raman by Using a Combination of Exfoliation and Flotation for Sample Pretreatment

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Moss can be used as a biomonitor for the determination of atmospheric pollutants such as heavy metals, nitrogen, and persistent organic components. As a result, local and time-dependent trends of these pollutions can be investigated. Up to now, it is unclear if moss can also serve as a biomonitor for atmospheric microplastic deposition. This study demonstrates the development of a method for identifying and quantifying microplastics in moss using Raman microscopy ( $\mu$ Raman) and thermal extraction-desorption gas chromatography mass spectrometry (TED-GC-MS). For sample preparation, microplastics on the moss surface were exfoliated with glass beads ( $d = 0.5$  mm). Furthermore, the exfoliated sample was sieved ( $< 1$  mm) and a flotation was performed using the recently published microplastics separator  $\mu$ SEP. The TED-GC-MS investigations showed the robustness and reproducibility of this kind of sample preparation procedure by performing microplastics recovery experiments. Also, chromatograms of high quality can be observed, which reduce the impact of matrix interferences on the identification and quantification of microplastics in a sample. The  $\mu$ Raman analysis indicated a high separation quality because of the possibility to perform a contrast-based particle identification on gold-coated filters. In addition, this method was applied to moss specimens collected from different sampling sites in Germany, which were part of the German moss survey 2020/2021. The moss survey is supported by the German Environment Agency (Research code: 3720632010). Within first investigations of these samples, different polymers, such as polyethylene terephthalate (PET) and polyethylene (PE) could be identified and quantified.

### 3.04.P-Mo174 Laboratory Testing of Hexachlorocyclohexane Retention in Moss as a Potential Biomonitor for the Surveillance of Water and Air Pollution

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Passive biomonitoring with mosses has emerged as a useful tool for the study of environmental pollutants, but its application in persistent organic pollutants (POPs) monitoring is limited and mainly focused on PAHs. This work aims to evaluate the capacity of two moss species to retain hexachlorocyclohexane isomers ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -HCH) from aqueous and aerial media to determine their potential use as biomonitors in HCH-contaminated environments. *Fontinalis antipyretica* Hedw. and *Sphagnum palustre* L. were selected because of their widespread use in aquatic and atmospheric pollution monitoring, respectively. Moss clones grown in photobioreactors and devitalised (oven-drying with a temperature ramp) were used. The biosorption of HCH from aqueous media was evaluated by a batch experiment of successive additions of a solution of HCH isomers (total concentration of 10.2 mg L<sup>-1</sup>, above solubility limit). Spiked moss suspensions ( $n = 5$ ) were left under agitation until equilibrium (24 h) and the analytes were determined in the liquid phase by ultrasound-assisted extraction (UAE) followed by gas chromatography-mass spectrometry analysis. Free-moss solution was used as control. The biosorption was calculated by the difference of the equilibrium concentration with the added concentration of HCH and sorption isotherm models were used for quantitative description of sorption processes. The adsorption of HCH from air was assessed by active indoor air sampling based on a solid phase extraction (SPE) system. The air was fortified with HCH isomers to obtain the spike level of 0.5 mg kg<sup>-1</sup> and then pumped through two serial samplers with sorbent: the first with moss tissues (or Florisil® as control) and the second, with Florisil®. Sampling was carried out in triplicate. HCH isomers in sorbent samples was quantified by UAE and gas chromatography-tandem mass spectrometry analysis. The biosorption was determined according to the recoveries achieved.



Data obtained from sorption experiment in aqueous media showed that *F. antipyrretica* bioaccumulated up to 4792 mg HCH per kg moss and a good fit to the Freundlich and Lineal models was achieved. Very low bioaccumulation from water was observed using *S. palustre*. In the data obtained from sorption experiment in air, recoveries ranged 89 – 129 % using *F. antipyrretica* and less than 70 % using *S. palustre*. *F. antipyrretica* had a higher retention capacity in both media, probably due to different cell wall composition.

#### **3.04.P-Mo175 Biological and Practical Implications of Trace Metal Accumulation in Devitalized Seaweeds**

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The biomonitoring of coastal waters with seaweeds is a method with potential to be a key tool to control trace metal pollution. However, further standardization and a better understanding of the uptake mechanisms of seaweeds are necessary to use this technique at a global scale. The objective of our study is to test the use of devitalized transplants of the brown algae *Fucus vesiculosus* to control trace element pollution, and to compare their accumulation patterns with those of fresh transplants to better understand the uptake pathways involved. To do this, we exposed material of *F. vesiculosus* picked up from an unpolluted site in six stations, five polluted and the collection site itself. In each place, four differently treated types of transplants were exposed: three treatments consisted of the three most apical dichotomies in mesh bags, the material was fresh in one of the types, dried in another, and boiled in the last. The fourth treatment consisted of whole thalli attached to stones by the basal part, as a control. Then we collected five transplants of each type after 4, 8, and 20, in addition to the day 0, and analyzed their concentration of ten trace elements. We found that devitalized transplants (dried and boiled) lost most of the material during the exposure period, in many cases being completely lost after 8 days, while the amount of material in fresh transplants (in bags and on stones) did not decrease. The concentrations of most elements (Al, V, Fe, Ni, Cu, Hg, and Pb) increased over time for all four types of transplants, but the increase was much more pronounced in devitalized transplants over fresh ones. Thus, the use of devitalized transplants for biomonitoring is limited to short exposition periods, but their quick uptake of most elements would make it possible. On the other hand, fresh transplants are more useful to get temporal representativity on a longer period, or insight on the impact of trace elements on organisms, as metal uptake in devitalized transplants is not comparable. Different factors can be the cause of the accumulation differences between live and devitalized seaweed, such as the exposure or modification of binding sites during the devitalization process, the inactivation of metal regulation mechanisms, or dilution by growth in live transplants. This experiment also indicates that elemental accumulation by seaweed is not mostly caused by active internalization of trace elements, but rather chemical adsorption.

#### **3.04.P-Mo176 Evaluating the Presence of Heavy Metals on Commercial Fish and Limpet Species in the Azores Region: First Approach Toward the Assessment of Seafood Safety for Human Consumption**

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The benefit of seafood consumption for human health worldwide is consensual. Nevertheless, the volcanic origin of the Azorean Region and the constant anthropogenic activities raise concerns regarding the quality status of fish and shellfish products consumed in the Region. Therefore, the academy and the government set out a monitoring programme to evaluate the contaminant levels in seafood species with high economic value. Besides ensuring the efficient protection of public health this programme aims to evaluate the “Good Environmental State” (Commission Decision 2010/477/UE) of the Azorean marine environment through the quantification of contaminants levels in demersal fish and mollusk species for human consumption.

The concentration of 4 heavy metals (Cd, Hg, As, and Pb) was determined in the muscle of 10 commercial species (*Phycis phycis*, *Phycis blennoides*, *Beryx splendens*, *Helicolenus dactylopterus*, *Conger conger*, *Pagellus bogaraveo*, *Beryx decadactylus*, *Mora moro*, *Sparisoma cretense*, *Patella aspera*) captured on the Azorean archipelago. Analysis of methylmercury is in progress in species that present the highest Hg content. The evaluation of this element is crucial for the measurement of consumers exposure levels to the most toxic fraction of Hg (Commission Regulation (UE) 2022/617).

Analysis was performed in dorsal muscle samples of each fish species and in all tissues of the limpets (*Patella aspera*). Metal analysis followed standard analytical methodologies, specific for each element: ICP-MS, GC, GC-MS, GC-ECD. Results were compared with the maximum levels set by the European Union legislation ((EC) No 1881/2006;(UE) 2022/617)). Results so far revealed high concentration levels of As and low concentrations of Cd and Pb in all demersal fish species. High Hg levels were found in splendid alfonsino and common mora. The common mora presented Hg levels above the maximum set by the EU legislation. Analysis are still in progress for european conger, blackbelly rosefish, greater forkbeard, parrotfish, and rough azorean limpet.

With this study, we will fill the gap of knowledge regarding the metal accumulation levels in demersal fish species and limpets, captured in the Azores Archipelago EEZ and also support policy decisions regarding the safe and conscious consumption of seafood.

#### **3.04.P-Mo177 Determination of Perfluoroalkyl Substances, Parabens, Bisphenols, and Metabolites of Phthalate Esters in Serum and Urine with APGC-MS/MS and UPLC-MS/MS**

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Perfluoroalkyl substances (PFASs), phthalate esters (PAEs), parabens, and bisphenols (BPs) are potential endocrine-disrupting chemicals. Biomonitoring of themselves or metabolites can provide crucial information for exposure and health risk assessment. This study developed and validated a method for determining 22 PFASs, eight PAE metabolites, six BPs, and four parabens in serum and urine. Serum samples were mixed with 1% formic acid in acetonitrile and passed through Waters Ostro plates. Urine samples were incubated at 37°C for enzyme deconjugation, then were mixed with acetonitrile and filtrated with Waters Sirocco plates.

Four fluorotelomer alcohols (FTOHs) and *N*-methyl perfluorooctane sulfonamide (*N*-MeFOSA) were separated on a J&W DB-5ms column and were analyzed with an atmospheric pressure gas chromatography coupled with a tandem mass spectrometer (APGC-MS/MS). The rest 35 analytes were determined with Waters ultra-performance liquid chromatography-MS/MS (UPLC-MS/MS) using UniSpray ionization; a Waters BEH C18 column and a Waters CORTECS column were used for the chromatographic separation. The method was applied to analyze 265 serum samples and 298 urine samples collected from 7- to 11-year-old children in 2018 in Taiwan.

The intra- and interday recoveries at three tested levels were higher than 80%, and the %RSD was lower than 20%; the limits of detection (LODs) were 0.12–1.12 ng/mL and 0.5–521 pg/mL for the assays of APGC-MS/MS and UPLC-MS/MS, respectively.

OA, PFNA, PFHxS, and PFOS were all detectable in serum samples at average concentrations of 2.20–5.14 ng/mL; 8:2 diPAP was also observed in all the samples with a mean of 89.8 ng/mL; the averages of FTOHs ranged from 0.51–1.19 ng/mL.

The average concentrations of PFBA, PFBS, and 6:2 PAP for urine samples were 29.4, 15.1, and 36.1 ng/mL, respectively; most average concentrations of PAE metabolites ranged from 23.0 to 104 ng/mL. The average BPA, BPF, and BPS concentrations were between 3.89 and 4.66 ng/mL. The positive rates of parabens were 92.6–100%, with the highest average concentration of methyl paraben at 419 ng/mL.

Levels of PFASs and BPs in samples positively associated with using plastic tableware and consumption of sea fish, oysters, and kelp. Concentrations of PAE metabolites and parabens in samples positively associated with using plastic tableware and lotion.

### **3.04.P-Mo178 A Human Biomonitoring-Based Study of Brazilian Lactating Women, Infants, and Children Exposed to Polycyclic Aromatic Hydrocarbons**

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Polycyclic aromatic hydrocarbons (PAHs) are highly lipophilic organic compounds formed during the incomplete combustion of organic matter from natural processes or by anthropogenic activities. PAHs are widely spread pollutants of public health concern, classified as endocrine disruptors, and have been listed as priority pollutants by the United States Environmental Protection Agency. Exposure to pollutants is even more critical during pregnancy, postnatal periods, and childhood. In these stages, some chemicals might induce genetic damage early in life and delay the epigenetic maturation of metabolic, endocrine, and neural pathways. Therefore, quantitatively determining these pollutants and their metabolites in biological matrices is essential for assessing human exposure; however, these biomonitoring approaches are limited in Brazil. Given the context, this study aimed to measure the levels of seven PAH metabolites (OH-PAHs) in urine ( $n = 400$ ) from Brazilian lactating women, infants, and children. The methodology used for quantification employed solid phase extraction and gas chromatography-mass spectrometry. Our results show that PAH metabolites were quantified in all samples. Besides, naphthalene was the major contributor to Brazilian exposure to PAHs, with a detection rate of 100%. 2-hydroxy-naphthalene (2OH-NAP) showed the highest concentration in lactating women and their infants, with a median of 0.97 ng/mL and 0.78 ng/mL, respectively. Whereas 1-hydroxy-naphthalene (1OH-NAP) presented higher levels (1.33 ng/mL) in Brazilian children, which may suggest exposure to the carbaryl insecticide since it is based on the high concentrations of 1OH-NAP. In this study, the ratio of 1OH-NAP/2OH-NAP in children was 1.78. 3-hydroxy-benzo[a]pyrene was the least detected metabolite, probably because this compound is primarily eliminated through feces. The Spearman correlation test showed high scores between the sum of OH-PAHs levels in lactating women and infants (0.62). In addition, it is possible to assess co-exposure between PAH classes in children. However, it is important to conduct studies that assess human exposure to multiple classes of environmental pollutants. This research should be a valuable tool to guide future regulatory actions that require a groundbreaking approach. Besides, future trials should be conducted to provide more generalizable results to other at-risk populations over time.

### **3.04.P-Mo179 Biomonitoring of Blood and Hair Samples to Assess Metals and Metalloids Exposure in Mother-Child Pairs from Delivery to 18 Month Old in a Cohort from the Population of Sevilla (Spain)**

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There is an increasing concern about the exposition during prenatal and early postnatal stages to environmental pollutants such as heavy metals and metalloids. Metals and metalloid are ubiquitous in the environment and pregnant women are exposed to them through their diet, lifestyle factors, or occupational and environmental sources. Among all population groups, pregnant women

and newborns are two of the most vulnerable groups since these substances can cross the placental barrier and access the fetus, even from the earliest times of gestation. After birth, the newborn continues to be exposed not only through the environment but also through their diet, including breast milk, having consequences on the health of the newborn. Numerous studies have shown associations between the prenatal exposition to some metals and an impact on cognitive, motor, and intellectual development of the child. Biomonitoring allows us to identify and eliminate possible sources of exposure, studying possible relationships between these pollutants and health problems.

The aim of this work was biomonitoring the levels of metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, and Zn) in a cohort of mother-child pairs in Seville (Spain). Samples of maternal and cord blood have been taken at the time of delivery, previous signing of the informed consent, and filling the epidemiological questionnaire. Hair samples from the newborns have been collected at 6, 12, and 18 months, being an ideal sample to determine metals, as these accumulate throughout human life, and because it is a noninvasive sample for infants. Samples were submitted to a microwave digestion process optimized to minimize the loss of volatile elements and ICP-MS was used to determine the content of metals. Results showed a correlation between most metals on blood samples of maternal and cord blood. A multivariate analysis of variance (*MANOVA*) was used to study the correlation between all hair samples on the newborn from month 6 to month 18. All values were inside normal ranges for the study population.

### **3.04.P-Mo180 Monitoring of Heavy Metals Levels from Blood in a Rural Population of Sevilla**

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Environmental pollution is a global problem today. The "One Health" concept, promoted by the EU, focuses on the interrelationship between environmental health and the health of humans and other animals. Uncontrolled industrial activity, aggressive fertilisation programmes, and even natural events such as fires or volcanoes release toxic substances into the environment. Among these toxic substances are heavy metals. The accumulation and transfer of these metals from soils, aquifers, or air to organisms cause serious damage to human and animal health. Metals such as Lead (Pb), Cadmium (Cd), Aluminium (Al), and Chromium (Cr) cause serious health problems such as neurological disorders, liver problems, cardiovascular diseases, and even kidney complications. Very high concentrations of these metals are being detected in rural areas, which were considered "clean areas."

The aim of this study was monitoring the concentration of Pb, Cd, Al, and Cr in the blood of a cohort of agricultural and nonagricultural workers in the region of Estepa in the province of Seville, southern Andalusia. The selection of participants was nonprobabilistic in a snowball sampling. A final sample of 100 men (age between 18 and 45 years) were asked to be part of this longitudinal study. In this case, 50 of them were classified as farmers group and the other 50 participants who work and live in the same rural environment were classified as nonfarmer group.

Samples were collected once a year during the harvest time in the Occupational Medicine Unit (Preventiam). Collected blood was refrigerated at 4 °C during transportation and immediately stored at -80 °C before analysis. Samples were submitted to a microwave digestion process optimized to minimize the loss of volatile elements. ICP-MS was used to determine the content of Cu. IBM SPSS software was used to perform the data analysis.

Preliminary data show that exposition prolonged of residents (agricultural worker or not) to heavy metals (Pb, Cd, Al, and Cr) leads to an increase in the blood level of these metals. Slightly lower levels were found in residents who did not work in the fields. These data should be carefully analysed, since the study has not yet been completed.

### **3.04.P-Mo181 Monitoring of Copper Levels from Blood in a Population of Olive Farmers from Seville (Spain).**

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Copper derivatives such as sulphate of copper have been commonly used in agriculture for disease control. In the case of olive, copper is the main pesticide authorized for the control of "Repilo," a fungal infection which causes great economic losses to the olive sector. Its long-term application leads to a copper accumulation in olive soils, together with the remaining traces accumulated in leaves, posing a risk to worker health. Although copper is an essential trace element, its prolonged indirect exposition is associated with serious health problems such as neurodegenerative diseases, hepatic disorders, atherosclerosis, cardiovascular diseases, or even renal disorders. Due to high temperatures in southern Andalusia, olive farmers are unable to wear personal protective equipment. In addition, these suspended particles containing copper could travel to nearby urban areas.

The main objective of this study was to monitor levels of copper in blood from a cohort of olive farmers from Seville (Spain). The selection of participants was nonprobabilistic in a snowball sampling. A final sample of 80 men (age between 18 and 45 years) was asked to be part of this longitudinal study. In this case, 60 of them, directly involved in collection of olives in the field, were classified as farmers and the 20 remaining participants who work and live in the same rural environment were classified as nonoccupational exposed (NOE) group.

Samples were collected during the olive harvest time in the Occupational Medicine Unit (Preventiam). Collected blood was refrigerated at 4 °C during transportation and immediately stored at -80 °C before analysis. Samples were submitted to a

microwave digestion process optimized to minimize the loss of volatile elements. ICP-MS was used to determine the content of Cu. IBM SPSS software was used to perform the data analysis.

Preliminary data indicate that exposition prolonged to copper affects not only farmers but also, to a lesser extent, people living in the same rural environment. These data should be carefully analysed, since the study has not yet been completed.

#### **3.04.P-Mo182 Maternal Urine and Amniotic Fluid Biomonitoring Using Electromembrane Microextraction (EME) to Assess Paraben Exposure in a Cohort from the Population of Sevilla (Spain)**

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Among all population groups, pregnant women are considered one of the most vulnerable due to many factors, including the increasing concern about exposition during prenatal stages to environmental pollutants such as parabens. These contaminants are a widespread group of endocrine disrupting compounds (EDCs), with confirmed transplacental passage. The usage of cosmetics, pharmaceuticals, and consumer products during pregnancy that may contain parabens has led to the need for monitoring surveys. To date, some works have demonstrated fetal exposure to parabens affects their development and subsequently adult health.

In this study, maternal urine and amniotic fluid samples were obtained at delivery from Spanish pregnant women after signing the informed consent to determine simultaneously their possible exposure to 7 parabens (methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and benzyl) and their main hydroxybenzoic acids metabolites (4-hydroxy, 3,4-dihydroxy, and 3,4,5-trihydroxy). Sample treatment was performed by EME, a simple liquid-liquid microextraction technique based on the use of an electric potential to achieve a selective extraction across an organic solvent known as supported liquid membrane (SLM). Polypropylene hollow fibers were used as support for the organic solvent. Analytes were extracted using a three-phase configuration applying the following conditions: donor phase pH 4 (10 mL), acceptor phase pH 13 (50  $\mu$ L), stirring rate 400 rpm, applied voltage 30V, extraction time 40 minutes and 1-octanol as SLM.

Samples were analyzed by ultra-high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-QTOF) on a Zorbax XDB C18 column (150 mm  $\times$  3.0 mm i.d., 3.5  $\mu$ m particle size) at 30  $^{\circ}$ C using 5mM ammonium acetate and methanol as mobile phase at a flow rate of 0.4 mL/min for 10 min under gradient elution. The detection was performed with negative ionization in an electrospray source at 1.5 kV capillary voltage, 120  $^{\circ}$ C for source temperature and 350  $^{\circ}$ C for desolvation temperature. The analyzed samples showed positive results for most of the selected parabens and their metabolites.

#### **3.04.P-Mo183 Human and Environmental Lead Exposure from Abandoned Lead Acid Battery Recycling Sites in Bangladesh**

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Lead is a potent neurotoxin and cases of lead poisoning evolving globally. About 800 million children worldwide have blood lead levels (BLL) at or above 5 micrograms per deciliter ( $\mu$ g/dL); half of them live in South Asia. Among many possible sources of lead poisoning, Used Lead Acid Battery (ULAB) recycling is one of the significant contributors to elevated BLLs. Here we explored the baseline data of a quasi-experimental study with a control group to assess the extent of environmental lead contamination within 700 meters radius of abandoned ULAB sites and the BLL status of children aged 0 to 12 years. Two hundred one children (151 from ULAB-exposed and 50 from ULAB-nonexposed sites) were surveyed and blood and environmental samples were collected with proper consent and precaution. Blood samples were analyzed using graphite furnace atomic absorption spectroscopy and environmental samples were analyzed using a handheld XRF (X-ray fluorescence) machine at icddr,b. The average BLL of the children living in the ULAB exposed area was 10.4  $\mu$ g/dL (SD: 6), where male children had higher BLL than females (11.3 vs. 9.2  $\mu$ g/dL). Ninety-nine percent of the children in the ULAB-exposed area had BLL more than 3.5  $\mu$ g/dL, compared to 78% children of ULAB-nonexposed children. According to WHO, BLL more than 5  $\mu$ g/dL requires medical attention, and we found an alarming 91% of ULAB-exposed children's BLL exceeded this value. Nevertheless, the ULAB-nonexposed group had 38% children whose BLL was more than 5  $\mu$ g/dL. The lead level of the ULAB exposed area's environmental samples was consistently higher than nonexposed area samples. The lead concentration of the most contaminated child play area soil sample was 4249 ppm (median 36 ppm). Height amount of lead detected in other environmental samples like floor dust, courtyard soil, and roadside dust are 389, 1088, and 773 ppm lead, respectively. Out of 147 turmeric powder samples tested, 11 were lead-contaminated. People living close to ULAB sites are at higher risk of lead poisoning due to the high contamination of surrounding soil and dust with lead compared to the ULAB-nonexposed group. However, the ULAB nonexposed group's BLL was higher than the US and other children of developed countries, which urges the further exploration of significant sources contributing to higher BLL among the general population of Bangladesh.

#### **3.04.V Application of Biomonitoring Approaches to Support Surveillance of Chemical Exposure in the Environment**

##### **3.04.V-01 Time Trends in PCB and PBDE Congeners and in $\Sigma$ PCBs and $\Sigma$ PBDEs Residue Concentrations in the Common Buzzard *Buteo buteo* in the Netherlands 1994-2020 in Relation to Restrictions on Chemicals Use.**

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EU chemicals regulation has introduced restrictions (bans and other risk management measures) to reduce the threats posed by the presence of persistent, bioaccumulative and toxic substances to human and wildlife health. In particular, restrictions have been introduced over recent decades on the use of many persistent organic pesticides (POPs).

Top predators such as raptors can be used as sentinels to assess the effectiveness of such restrictions on reducing residue concentrations in the environment. The common buzzard *Buteo buteo* is widely distributed in Europe and has been suggested as a suitable species to systematically monitor trends in terrestrial wildlife exposure to contaminants in the environment at national and pan-European scales. To date, limited use has been made of such systematic biomonitoring to assess the efficacy of restrictions in reducing wildlife exposure to chemicals at these scales.

We carried out biomonitoring of 31 PCBs and 23 PBDEs (including decaBDE) in buzzard livers at country and pan-European scales to examine whether restrictions have led to reduced exposures over time. In this poster, we report on the country scale study for the Netherlands.

We used 64 buzzard livers from birds found dead in ten Dutch provinces over the period 1994-2020. We analysed these livers for PCBs and PBDEs using GC-MS. Time trends for the numbers of PCB congeners and of PBDE congeners, and for sum of PCBs residues ( $\Sigma$ PCBs) and sum of PBDE residues ( $\Sigma$ PBDEs) were modelled using linear models, integrating year of sampling, age, sex and location (province) as variables. Significant effects of these variables on the compositions of PCBs and PBDEs in livers were assessed by redundancy analysis.

Our initial findings show a significant difference in PCB composition and  $\Sigma$ PCBs, but not in PBDEs, between the province of Drenthe and other provinces. Age was not found to be a significant factor in either  $\Sigma$ PCBs or  $\Sigma$ PBDEs. We observed a declining trend over time in the number of PCB congeners,  $\Sigma$ PCBs and  $\Sigma$ PBDEs in males, but not in females. These findings suggest that the effectiveness of regulatory restrictions in the buzzard might vary with sex and location, but not age. Interpretation of these findings should take into account any data bias arising from opportunistic sampling and sample size.

We discuss the implications of our findings for the further application of biomonitoring in Europe to assess the effectiveness of regulatory restrictions.

#### **3.04.V-02 Comparison of the Accumulation of Atmospherically Deposited Metals and Nitrogen in Mosses Collected Throughout Germany with Data from Emission Inventories and Deposition Models**

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Emission inventory data are used for modelling and mapping atmospheric deposition, and mosses are used for recording the bio-accumulation of atmospheric deposition. Crucial questions are, whether emission, exposure and modelled deposition data are correlated. To answer them, we used data from the German national emission register, latest data derived by deposition modelling and data derived by moss surveys. The comparison of the moss data determined between 1990 and 2020 showed a significant decrease of metal bio-accumulation. Nevertheless, contrary to emission data, intermediate increases of metal accumulation compared to the preceding moss survey was observed between 2000 and 2005 and from 2015 until 2020. Trends in nitrogen medians over the last three campaigns, 2005 to 2020, show that nitrogen medians decreased by -2% between 2005 and 2015, and increased by +8% between 2015 and 2020. The differences are all nonsignificant and not consistent with emission trends. Results of most recent modelling of atmospheric concentration and deposition of the metal elements Cd, Hg and Pb were compared with the results of technical measurements and moss surveys. The modelling results with status 2020 have a higher spatial resolution of 0.1° x 0.1° than the modelling results valid up to then (50 km x 50 km). This is associated with partly slightly higher correlations between the findings of the modelling and those of the moss monitoring. For a statistically adequately deepened analysis and evaluation of the high-resolution modelling results it is particularly important to link the exposure data derived by deposition modelling, by technical deposition sampling and by moss surveys with information on the receptors in terms of ecosystem types. This would be a step forwards to a more differentiated risk assessment of impacts on ecosystems due to atmospheric metal deposition.

#### **3.04.V-03 Pollution Induced Alterations in the Motility of Mussel Hemocytes: An In Field Study**

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In the last decades, effect-based methodologies such as exposure/effect biomarkers in sentinel organisms have known an increasing interest in environmental biomonitoring and assessment. The present work aimed to study pollutant-induced alterations in the motility of hemocytes in the bioindicator organisms *Mytilus galloprovincialis* in view of ecotoxicological biomonitoring and assessment application. Hemocytes are motile, phagocytic cells that represent the key elements of innate immunity in bivalves. Motility is an intrinsic characteristic of these cells since the first phase of their immune response is represented by their recruitment and migration to the site of infection.

The study was carried out on native *M. galloprovincialis* collected from 6 sites along the coast of the Salento Peninsula (Italy), two control sites, and four impacted sites characterized by the presence of an industrial plant close to Brindisi town (Apulia, Italy) and a thermoelectric central. Hemolymph was withdrawn from the posterior adductor muscle sinus of mussels, it was diluted in the sampling syringe 1:1 with filtered seawater (0.2µm filters) and then added in the wells of a 96-well flat bottom polystyrene TC-treated microplates. Hemocytes were allowed to adhere for 30 min at 15 °C. Then, the cells were imaged by time-lapse microscopy, at a rate of 1 image every 2min for 20 min using Cytation 5 cell imaging multimode reader. Cell tracking was performed on single cells; velocity, migrated distance, and directionality of migration were quantified.

Results showed a statistically significant decrease in the velocity and migrated distance of hemocytes of mussels sampled in all the impacted sites with respect to control sites; moreover, alterations in the directionality of migration were also detected. The use of the neutral red retention assay on the same hemolymph sample allowed the detection of lysosomal membrane stability destabilization and in turn the presence of a stress syndrome in the specimens coming from the impacted sites.

In conclusion, obtained results provide a novel approach to analyzing hemocyte activity and open the perspective for the use of cell motility as a cellular function sensitive to exposure to chemical pollution in the environment.

### **3.05 (Bio)Degradation and Persistence of Chemicals - New Perspectives and Developments**

#### **3.05.P-Th162 Development of a High Throughput Method for Screening of Readily Biodegradable Chemicals**

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Substitution of hazardous chemicals with safer and greener chemicals is part of the EU strategy for a nontoxic environment and a circular economy. Part of the definition of being safe and sustainable is that the chemical is not persistent, meaning that it will biodegrade in the environment. However, current standard test methods are laborious and not suited for high throughput screening of chemicals because of both the required volume of the test systems, and the limited possibility of automation of the analytical methods. A high throughput method should preferably be in a miniaturized system to minimize footprint in the laboratory, be based on an analytical method suitable for automation, and based on a generic parameter.

The aim of this study was to develop a high throughput biodegradation test for nonvolatile and water-soluble organic chemicals based on growth of bacteria as an indicator of biodegradation, measured by flow cytometry (FCM). A set of eight reference chemicals were included in the test method development and four sources of environmental bacterial communities. To allow for high throughput and automation, incubation of environmental inoculum with test chemicals were performed in deep 96-well plates which could be sampled directly by a multipipette to 96-well plates that were compatible with FCM.

In most cases, the positive controls aniline and sodium benzoate gave significant increased bacterial concentrations in some or all concentrations of added test chemical. However, the rest of the reference chemicals did not, or only to a very limited extent, induce significantly increased growth of bacteria. This is likely due to the stringent test conditions of this set up with only one ml volume of test medium, low bacterial number, and these chemicals being only moderately biodegradable. However, since both positive controls induced significant growth compared to the blank control in most experiments, the method showed potential for being used to identify readily biodegradable chemicals.

A total of 24 96-well plates with different inoculum variations were included in the experiment, with each well plate containing eight test chemicals at three different concentrations in experimental triplicates. Thus, a total of 576 experiments were run in triplicates on one instrument by one person over a six-week period. This demonstrated the high throughput potential for this method that should be included in the toolbox for developing new green chemicals.

#### **3.05.P-Th163 Investigation into the OECD 309 Surface Water Mineralisation Test – Exploring Impacts of Sample Collection and Storage, Experimental Factors, and Reference Compounds**

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Biodegradation is an important process for the removal of several environmental organic contaminants and is a major determinant of both chemical persistence and chemical exposure. Guidelines for chemical hazard assessment in certain regional and global regulations, such as the European chemicals regulation REACH, set thresholds for persistence in environmental compartments (e.g., degradation half-life in water) that need to be evaluated quantitatively. Furthermore, to assess chemical exposure, quantitative estimates of degradation half-lives are required for environmental media that influence the exposure of target organisms.

In this ongoing work, the OECD 309 Surface Water Mineralisation Test is investigated in detail by providing an in-depth assessment of inoculum quality and viability; identifying and validating more relevant reference substances that better reflect the vital status and composition of the inoculum and providing a data-based evaluation of the test robustness and applicability at the new mandated test temperature (12°C in freshwater/9°C in seawater). The data obtained will form a basis for providing improved

guidance on surface water sample collection, storage, and treatment. A suite of quantitative and qualitative tests (as MPN/CFU, respirometric analysis, 16S rDNA microbiome analyses) that further demonstrate inoculum status and guidance on the test validity criteria will incorporate detailed information on selected reference chemical biodegradation rates at different temperatures. Besides the well-known reference substances sodium benzoate and aniline as a fast-degrading substance, there is also a need for reference substances with a slower degradation.

These improvements will be based on the principal theme of characterising the relationship between inoculum diversity, viability, and performance of biodegradation with more appropriate new reference substances. Characterization of the bio-physio-chemical water sample diversity may be important to resolve inoculum variations between seasons and changes during storage, and to secure the chance of competent cells in a biodegradation process. Studies of microbial community structure and succession analyses have also proven to be important for a more comprehensive understanding of biodegradation processes.

### 3.05.P-Th182 Biodegradation Testing of Constituents of Natural Complex Substances

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The use of Naturals as ingredients in perfumery has a long history. In the pursuit of environmental sustainability, using renewable materials rather than synthetic ingredients derived from petrochemical feedstock, is of increasing interest. At the same time the question of the environmental impact of fragrances is central – namely the biodegradability of ingredients.

Some of the Naturals used in perfumery are complex in chemical composition, consist of a high number of different chemical constituents, and are therefore commonly designated as Natural Complex Substances (NCS). OECD screening tests for ready biodegradability are not generally applicable for complex mixtures containing different types of chemicals. A case-by-case evaluation has to take place on whether a biodegradability test on such a complex mixture would give conclusive information regarding the biodegradability of the mixture as such or whether instead an investigation of the biodegradability of individual components of the mixture is required.

While composition information is available on a number of NCS, this information may not be sufficiently detailed. In such cases gathering additional information on the composition of the NCS is the first step of the biodegradability assessment. Ideally all constituents  $\geq 1\text{wt}\%$ , and at least 80wt% of the NCS are identified - it is impractical (even impossible) to identify all minor components in NCS. However, the minor constituents in the remaining unknowns of a given NCS are generally related to the identified constituents and hence expected to have similar biodegradation profiles.

The next step is gathering information on the biodegradability of the known individual constituents. If data on constituents are not available, data need to be generated for these “missing” constituents. This may in some cases include the isolation of the individual constituents to be tested from the NCS or involve the testing of more readily available representative molecules.

In this presentation, new biodegradation results for key constituents of fragrance NCSs will be presented. Case studies will also be used to show how this new data has allowed the completion of the biodegradation assessment of NCSs using the constituent approach where before crucial information was missing. For some Naturals specific constituents / or the Natural as such were tested to complete the assessment, demonstrating that the two approaches (constituent and whole substance) are complementary.

### 3.05.T-01 Regulatory Landscape for Persistence Assessment in PBT/vPvB and PMT/vPvM Identification Under REACH and CLP

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Many actions have been taken to clarify, improve, and harmonise hazard assessment of substances in EU. “One substance, one assessment” is one of the key elements in the Chemical Strategy for Sustainability (CSS) adopted by the European Commission on 14<sup>th</sup> October 2020. EU Commission considers CLP Regulation (EC) No 1272/2008 as one of the key regulatory “tools” to centralise the “One substance, one assessment”. Identifying substances as PBT (persistent, bioaccumulative, toxic), vPvB (very persistent, very bioaccumulative), PMT (persistent, mobile, toxic), or vPvM (very persistent, very mobile), was considered necessary to be introduced as new hazard classes and criteria into CLP Regulation. Furthermore, the REACH ((EC) No 1907/2006) Annexes VII-XI on hazard information requirements were revised to clarify the obligations of registrants regarding the submission of information. To inform and integrate on the scientific and technical advances in the assessment of PBT/vPvB substances, ECHA has launched revision and subsequent consultation of the respective ECHA Guidances on IR&CSA, Chapters R.11, R.7b and R.7c. Persistence is a fundamental property of a substance to be considered in environmental hazard, fate, exposure, and risk assessments across different regulatory frameworks. Persistence assessment is also the first step in the assessment of PBT/vPvM and PMT/vPvM properties. This presentation will provide an overview of the regulatory actions taken to improve identification and regulation of persistent substances and recent scientific advances in PBT/vPvB and PMT/vPvM assessments.

### 3.05.T-02 Impact of Different Sterilisation Techniques on Sorption and NER Formation of Test Chemicals in Soil

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Standard OECD tests are used to generate data on the degradation (OECD 307) and sorption (OECD 106) of test chemicals in soil. In degradation testing, data on abiotic degradation using sterile samples is utilised to investigate any losses due to abiotic processes. In the context of sorption and nonextractable residues (NER) formation, data from sterile samples are often used to interpret results and findings of nonsterile samples. Generally, sterile samples are prepared by sterilisation techniques using autoclaving, gamma-radiation, and soil poisoning (adding sodium azide). To ensure the comparability of the results obtained from sterile and nonsterile samples, the influence of the sterilisation should be minimal. However, studies have shown that sterilisation techniques can influence physical and chemical properties of soil and can result in different sterilisation efficiencies. In this study, we aim to further investigate 1) the impact of different sterilisation techniques on sorption and NER formation behaviour of chemicals in soil and 2) cross compare the results obtained on sterilisation efficiencies using traditional viable plate count (VPC) methods with the RNA (cDNA) molecular approaches. For this study, two different reference soils were used: I) 01-A with low organic carbon (OC) (0.93%) and II) 03-G with high OC content (3.01%). Soil samples were sterilised by autoclaving, gamma-radiation and soil poisoning with 1% (W/W) sodium azide. Two sets of tests were performed using the sterilised soil samples: 1) OECD 106 (tier 1-2), where sorption behaviour of test items was compared by calculating adsorption kinetics and  $K_d$ -values in sterile and non-sterile soils and 2) OECD 307 (only sterile sample), where impact of sterilisation techniques on sterilisation efficiencies and NER formation was studied. For OECD 307 test,  $^{14}\text{C}$ -labelled phenanthrene and bromoxynil were used, whereas for OECD 106 test nonlabelled phenanthrene and atrazine were used. The results from VPC and molecular approaches suggest autoclaving to be the most effective sterilisation technique. OECD 307 (only sterile sample) results showed differences in NER formation of the test chemicals, which could be due to inefficient sterilisation and/or change in soil phys.-chem. properties. OECD 106 (tier 1-2) results suggest that autoclaving of soil did not considerably affect the sorption behaviour of the test chemicals. The study is still ongoing.

### 3.05.T-03 The Relationship Between Composition and Environmental Degradation of Poly(Isosorbide Oxalate) (PISOX) Copolyesters

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To reduce the global CO<sub>2</sub> footprint of plastics, bio- and CO<sub>2</sub>-based feedstock is considered the most important design feature for plastics. Although reuse and recycling are the most desired end-of-life options for plastics, biodegradability will become very important when waste collection is not available/viable or to avoid endless accumulation of waste plastics into the environment. Oxalic acid from CO<sub>2</sub> and isosorbide from biomass are interesting rigid building blocks for high Tg polyesters. A family of novel fully renewable (bio- and CO<sub>2</sub>-based feedstock) poly(isosorbide-co-diol) oxalate (PISOX-diol) copolyesters was recently developed. We systematically investigated the effects of the composition on biodegradation at ambient temperature (25 °C) in soil for PISOX copolyesters by measuring CO<sub>2</sub> evolution. All copolyesters mineralised completely within 180 days (>80%; faster than cellulose as reference) except one composition with the cyclic diol CHDM (1,4-cyclohexanedimethanol) as second diol. Oxalate esters were shown to favour fast biodegradation independent of the type of the noncyclic co-diol. Moreover, their relatively fast degradability results from facile nonenzymatic hydrolysis of oxalate ester bonds (hydrolyse completely within 180 days). Thus, partially replacing oxalate by terephthalate units increased the resistance of the polymer to hydrolysis as well as to biodegradation. We demonstrated the variation in biodegradability of PISOX copolyesters by their lag phase, ranging from 1 to 10 weeks and by the time to reach complete biodegradation, typically all in a matter of months for compositions with noncyclic second diol comonomers. This work shows the potential for tuning the composition of PISOX copolyesters, for optimizing the resulting properties (thermal-, mechanical-, barrier-, hydrolysis- and biodegradability) to target certain applications, such as controlled release for fertilizers, films and rigids for packaging, 3D printing, etc.

### 3.05.T-04 Influence of Season on Biodegradation Rates in Rivers

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There is evidence that biodegradation rates in the environment have considerable temporal variability, which can have a major impact on overall chemical fate. However, our quantitative knowledge of the rate of biodegradation during different seasons remains limited. Existing data are fragmented and often derived from tests with questionable environmental relevance. The present study therefore has two main objectives: (i) To obtain insights into the influence of season on biodegradation rates of a broad range of contaminants in surface water through a modified OECD 309 test with better environmental relevance; (ii) to explore whether the temporal variability of the biodegradation rates can be described using proxies for microbial biomass. We performed a modified OECD 309 test with water and sediments from up- and downstream of wastewater treatment plants in 2 Swedish rivers (Hågaån, Knivstaån) during 4 seasons (winter: early March, 4 °C; spring: May, 17 °C; summer: August, 19 °C; autumn: October, 11 °C). An aqueous mixture of 129 compounds was spiked to a concentration of 1 µg L<sup>-1</sup> each and their dissipation was followed over a 10-day incubation. The total organic carbon content of the sediment and the cell density in the water and sediment will be measured for both field samples and samples collected at the beginning and the end of each experiment. These measures will be used as viable microbial biomass proxies to test whether they can explain the variability of biodegradation rates across seasons. Initial results show high seasonal variability of  $k$  for some chemicals in both rivers. We expect the evaluation of the full dataset will further our understanding of the seasonal variability of biodegradation rates.



### 3.05.P (Bio)Degradation and Persistence of Chemicals - New Perspectives and Developments

#### 3.05.P-Th164 Dealing with Scarce or Ambiguous Information in Persistence Assessment – Two Perspectives

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Characterizing the environmental degradation behavior of chemicals is a pivotal element of chemical hazard and risk assessment. The EU Chemicals Strategy for Sustainability will further reinforce this because many thousands of chemicals will have to undergo a persistence assessment in this context. Two situations may frequently be encountered in evaluating the degradation behavior. First, existing information is ambiguous and, hence difficult to interpret. Second, information from simulation tests is unavailable (as is the case for thousands of substances). Weight-of-evidence approaches have been proposed for decision-making for those cases. Using the current guidance on persistence assessment of the European Chemicals Agency as a starting point the present contribution evaluates the usefulness of the weight of evidence approach and explores options for refinement. These options are discussed in the light of ease of application, range of substance covered, degree of precaution, and scientific soundness. An academic and an industry perspective are given.

#### 3.05.P-Th165 Biodegradation Assessment of Data Poor Substances: Case Studies Exploring a Weight of Evidence Approach

**Marlies Berghem**, **Thorsten Wind**, **Sven Langhans** and **Johannes Tolls**, **Henkel AG & Co, KGaA, Germany**

The introduction of the EU Chemicals Strategy for Sustainability will further increase the regulatory relevance of the persistence characterization of chemicals. It is foreseen that chemicals will have to be classified into two new hazard classes in the EU, 'persistent, mobile, and toxic' and 'very persistent, very mobile'. Persistence characterizations may be needed for some 7000 to 8000 chemicals (out of 12 500 chemicals) that are registered under the EU chemicals legislation REACH. Based on the currently available information these chemicals cannot be regarded as biodegradable based on results from ready or inherent biodegradation tests. Neither has their persistence been assessed based on simulation tests. These are highly costly and have a long duration. Hence, there are practical issues that may impede a rapid persistence characterization for thousands of chemicals. Those may be overcome by evidence, which can be considered sufficient to qualify a substance as 'principally degradable'. In this contribution we present the development of such evidence in several case studies. The evidence considered includes kinetics information from biodegradation screening tests, the expected formation of stable metabolites, structural similarities with chemicals with well-established degradation behavior, etc. The case studies demonstrate the integration of pieces of evidence into a 'principally biodegradable' evaluation.

#### 3.05.P-Th166 An Integrated Tool for the Screening of Fate, Persistence, and Long-Range Transport of Organic Chemicals

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Potential risks to human or environmental health caused by chemicals that are very persistent in the environment are poorly reversible. For chemicals with the potential to undergo long range transport (LRTP) such harm is not spatially contained. Prioritizing chemicals with respect to persistence (P) and LRTP is therefore often an important consideration in chemical assessment. P assessment is typically accomplished by comparing kinetic (bio)degradation data to bright-line pass/fail criteria while LRTP is often quantified by metrics such as characteristic travel distances (CTDs) or a transfer efficiency (TE). Other metrics to estimate P and LRTP (e.g., Overall Persistence (P<sub>OV</sub>) or the emissions fractions) exist and should be integrated in a Weight of Evidence (WoE) assessment framework. With the aim to establish a multimedia framework for the screening for P and LRTP, we designed the Fate and Persistence Estimation & Simulation Tool (F-PEST) and implemented it in the Exposure And Safety Estimation (EAS-E) Suite platform ([www.eas-e-suite.com](http://www.eas-e-suite.com)). F-PEST assembles information on chemical partitioning, emission, degradation, and environmental fate and transport for the prioritization and screening-level assessment of neutral and ionizable organic chemicals (IOCs). F-PEST input parameters such as chemical partitioning and distribution ratios and degradation half-lives in different environmental compartments are auto-parameterized from the EAS-E Suite database or predicted by built-in Quantitative Structure Activity Relationships (QSARs). These properties are then used in a mass balance model of fate in a multimedia environment that calculates the relative chemical mass distribution between, and the chemical activities in, the environmental compartments to identify media of concern. In addition to P<sub>OV</sub>, CTDs and TE, F-PEST estimates the three emissions fractions (i.e., dispersion, transfer and accumulation), thereby enabling a comprehensive P and LRTP assessment. A set of libraries reflecting different regional-scale environmental conditions is available to easily parameterize the system. Specifically, simulations can be run with the default conditions of the "OECD Pov and LRTP Screening Tool" or with sets of conditions adopted from other models (e.g., EQC, RAIDAR, EUSES) that are representative of different geographical areas (e.g., temperate North America, Europe). A case study with selected chemicals is presented to demonstrate the integrated framework.

#### 3.05.P-Th167 The Persistence Assessment Tool (PAT): Implementing a Methodology for Data Quality Evaluation and Weight of Evidence in Persistence Assessments

**Christopher Hughes<sup>1</sup>**, **Megan Griffiths<sup>1</sup>**, **Simon Cook<sup>1</sup>**, **Dik van de Meent<sup>2</sup>**, **John Parsons<sup>2</sup>**, **Delina Lyon<sup>3</sup>** and **Amelie Ott<sup>4,5</sup>**, (1)Ricardo Energy & Environment, United Kingdom, (2)Association of Retired Environmental Scientists ARES, Netherlands, (3)Concawe, Brussels, Belgium, (4)Cosmetics Europe, Belgium, (5)International Collaboration on Cosmetics Safety

Chemical persistence plays a key role in determining environmental exposure making it an important component in risk assessment and regulation. Recently, interest in chemical persistence has increased significantly. Persistence assessment in the regulatory context involves comparing chemical degradation half-lives to set criteria for different environmental compartments (water, sediment, and soil). Other information is also relevant for assessments (e.g., biodegradation screening tests, non-standard experiments, QSARs, field data, etc.), and should be considered following a weight of evidence approach. Implementation challenges remain in persistence assessments, particularly relating to guidance around the evaluation of data quality, and the weight-of-evidence determination. In addition, there are issues for substances whose properties render them difficult to evaluate using standard methods.

To address these challenges, a software tool – the Persistence Assessment Tool (PAT) – has been developed to support the evaluation of persistence under regulatory frameworks such as EU REACH. This tool provides clear guidance and structure to evaluate data quality, and a quantitative weight-of-evidence (qWoE) methodology to process the information input and calculate persistence conclusions in line with regulatory guidance. The PAT is applicable to all substance types and provides specific features to account for difficult and complex substances. Various options for customisation of the methodology are included to adapt assessments to specific regulatory frameworks and purposes. In addition a multimedia fate model, SimpleRisk4PAT, is included to optionally calculate overall persistence (P<sub>OV</sub>), allowing for additional potentially important environmental fate processes to be taken into account. The PAT aims to support robust, consistent and transparent decision-making for persistence assessment. There is a need for stakeholder input to support further validation, consensus-building and uptake of the methodology.

### 3.05.P-Th168 Persistence Revisited – Do Substances of Equal Degradation Half Life in Water Have Equal Lifetimes in the Environment?

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In Europe, under the REACH regulation, chemical substances with unacceptably long lifetimes in the environment cannot be used without restriction. Criteria to assess whether chemicals are “P” are, like in most international regulatory frameworks, based on comparison of single-media half lives with chosen cut-off criteria, silently assuming that chemicals with half lives that exceed the chosen criteria have lifetimes in the environment that are unacceptably long. However, criteria for (un)acceptability of lifetimes of chemicals in the environment. What is more, it has not even been decided how “lifetime in the environment” is to be measured. Environmental scientists in Europe and North America have proposed multimedia fate model based overall half life at steady state (P<sub>ov</sub>) as a useful measure of lifetime in the environment. Stroebe (2004) has warned against this on theoretical grounds, because P<sub>ov</sub> and single-media half lives tend to measure different things, so that exceedance of single-media half lives cannot be taken as evidence of unacceptability of lifetime in the environment.

We have tested whether QSAR-derived half life in water can be used as predictor of calculated P<sub>ov</sub>. To this end we have used EUSES to calculate overall persistences in the environment of 1977 REACH-registered organic substances, and compared these with QSAR-estimated degradation half lives in water of the same 1977 substances. A plot of the results will be presented at the poster. It clearly shows that substances with equal half lives in water may have greatly different P<sub>ov</sub>. This result confirms the theoretical work of Stroebe (2004): substances with short half lives in water may have long environmental lifetimes.

### 3.05.P-Th169 The Importance of Accurate Measurements of Nitrification in Biodegradation Tests

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Nitrification is an important process in soils, wastewater treatment plants, and in general in the ecosystem because it completes the mineralization of organic nitrogen. The conversion of nitrogen into nitrate is done in two steps by two different groups of microorganisms, the first step is the conversion of ammonium into nitrite ( $\text{NH}_4^+ \rightarrow \text{NO}_2^-$ ) and the second is the rapid oxidation of nitrite into nitrate ( $\text{NO}_2^- \rightarrow \text{NO}_3^-$ ). These biochemical reactions are of high importance in biodegradation testing of organic nitrogen compounds. Those that are susceptible to biodegradation will release nitrogen atoms as ammonium and, if nitrification subsequently occurs, ultimately in the form of nitrite and/or nitrate. Accurate measurement of nitrification is essential in biodegradation testing because serious errors can arise in the determination of the percentage of biodegradation by oxygen uptake (i.e., in respirometric OECD methods 301F, 301D, 302C, etc.) if the observed oxygen uptake is not appropriately corrected by the amount used in oxidizing ammonium to nitrite and ultimately nitrate. Simple methods for the measurement of nitrite and nitrate in solution may include coloured test stripes, which are fast but of low accuracy, and/or colorimetric methods, which are more accurate but require a previous derivatization of nitrogen into an UV absorbent species before analysis. In this poster, we propose the use of a simple and optimized method for the measurement of nitrate at the end of respirometric biodegradation tests by using a highly sensitive Ion Selective Electrode (ISE). After an initial calibration, the use of an ISE allows a fast and accurate determination of nitrate in a complex matrix (salt medium + activated sludge). An optimization of the method was also made to determine the right ionic strength needed in solution when measuring low levels of nitrate (< 2ppm). Quantifying the exact values of nitrate in the solution allows us to calculate a weighted theoretical oxygen demand of the substance by using the proportions of nitrate and ammonium. This calculation yields a more accurate determination of the overall percentage biodegradation of the test

substance and avoids over- or underestimations. Some examples of calculation of biodegradability with this approach will be presented.

### **3.05.P-Th170 Evidence of Nitrogen Limitation in Manometric Respirometry Tests for Ready Biodegradability**

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Standard methods for ready biodegradability assessment employ stringent ‘fail-safe negative’ conditions (unadapted inoculum, test item is the sole source of C for microbial energy and growth needs, high substance:biomass ratio). All use an aqueous medium containing inorganic salts intended to support microbial growth requirements for the duration of the test. The current manometric respirometry test guideline of the Organization for Economic Cooperation and Development (OECD TG 301F) has its origins in a 1983/4 EC ring-test that *i.a.* gave rise to a proposal to lower the medium N content – not to ensure test stringency and minimize the potential for false-positive test outcomes, but as a technical fix to limit the potential for nitrification to confound oxygen uptake measurements. The fix was adopted without further validation or assessment of impact on test outcomes. We present evidence of N limitation in tests performed with a multiconstituent derivative of *Eucalyptus citriodora* oil. Rate and extent of mineralisation under OECD 301F conditions were reproducibly influenced by the inorganic N content of the test medium. There are implications for the rate constant for removal in STP and consideration of persistence in the EU regulatory framework.

### **3.05.P-Th171 The Importance of Data Relevance Criteria for Regulatory Use of Nonstandard Test Data for Persistence and Bioaccumulation Assessments**

**Leslie J. Saunders<sup>1</sup>**, Delina Lyon<sup>1</sup>, David Saunders<sup>2</sup>, Louise Camenzuli<sup>3</sup>, Sandrine Sourisseau<sup>4</sup>, Christopher Hughes<sup>5</sup> and Emma Pemberton<sup>5</sup>, (1)Concawe, Brussels, Belgium, (2)Shell Global Solutions, Netherlands, (3)ExxonMobil, Belgium, (4)TotalEnergies, France, (5)Ricardo Energy & Environment, United Kingdom

Regulatory assessments of persistence, bioaccumulation, and toxicity (PBT) rely on available data, either generated directly by registrants or from other scientific sources. Following evaluation, substances meeting PBT criteria are identified as Substances of Very High Concern (SVHC), which can have far-reaching repercussions (see Borchert et al., 2022). While standardized test methods (e.g. OECD/ISO) may be used to generate data for such assessments, these data may be unreliable as these methods are not suitable for all types of substances with varying physicochemical properties. In such cases, modified or nonstandard test methods are often used to accommodate substance-specific issues (e.g., hydrophobicity, volatility, UVCBs). Additionally, as per the Chemical Strategy for Sustainability under the EU Green Deal, it is expected that use of non-standard data including NAMs will become more common in regulatory decision making. However, data generated in nonstandard tests may be variable and their analysis complex which can inevitably pose challenges to determine which studies will achieve regulatory acceptance. Hence, more systematic approaches to data quality assessment are needed to support greater consistency and transparency in PBT assessments (Pemberton et al. 2022. SETAC Copenhagen [We125]). The quality of standard and nonstandard data is typically assessed for relevance and reliability. Approaches for reliability evaluation have evolved over time, becoming more systematic with available scoring criteria (e.g., Klimisch). However, there is currently much less consensus on data relevance scoring, which may introduce bias and inconsistencies in the assessments. This is particularly true for P&B evaluations, where there are currently no agreed upon relevance criteria. In the present study we reviewed multiple studies used in previous evaluations and the wider scientific literature to determine the types of P&B data used in regulatory decision making. We targeted studies performed with difficult-to-test substances (i.e., UVCBs), since evaluations of these substances often rely on nonstandard data. Using this information, we identified relevance criteria that are being used in practice for P&B data with the goal of informing future study methodologies that would result in more acceptable data for regulatory evaluation.

### **3.05.P-Th172 OECD 309 Study – What is the Role, Purpose, and Relevance in EU Risk Assessment?**

**Derek Wallace**, ERM Regulatory Services Limited, United Kingdom

The evaluation of the fate and metabolism of active substances applied in the environment are prescribed by Commission Regulation (EU) No 283/2013, which outlines the requirement to define behaviour in water/sediment systems (OECD 308) and open-water bodies (OECD 309). Using the information generated whilst conducting the key OECD guideline studies, the exposure of active substances in surface water can be modelled using FOCUS Steps 1-4. However, in the FOCUS Surface Water Generic Guidance, the characteristics of typical edge-of-field water bodies are defined and broadly grouped as “Pond”, “Stream” and “Ditch” scenarios and there is no assessment or parameterisation of an “open lake” scenario.

In order to mimic exposure to “open water bodies”, which denotes degradation with a very low sediment component, the OECD 309 study is required. Typically, results in the OECD 309 study are variable, characterised by slow degradation with evident lag phases, plus the potential for identification of novel metabolites not typically observed in the OECD 308 study. This creates the potential requirement to identify, synthesise and characterise these metabolites in order to perform a risk assessment; in particular driven by the lack of an exposure scenario and the tendency to assume extremely conservative ecotox endpoints. Given that the nature of exposure to an OECD 309 study explicitly describes a significant volume of water and that FOCUS SW Step 1-4 describe edge-of-field exposure scenarios of water bodies not fitting this description, the requirement to conduct an assessment for unique OECD 309 metabolites against FOCUS PECSW is overly conservative.

In conclusion, the role and application of the OECD 309 study in EU Crop protection risk assessment is reviewed against the original definitions of edge of field water bodies in FOCUS Surface Water. Considering the definitions of the water bodies, the relevance and appropriateness of applying observations from the OECD 309 study requires discussion.

### 3.05.P-Th173 Pitfalls and Potential of the OECD 309 Test

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The assessment of persistence is of growing importance in chemical regulation. With the introduction of a persistent, mobile and toxic (PMT) hazard class, many more chemicals will be subject to persistence evaluation under REACH. The prioritized test for assessing persistence under REACH is OECD 309, a simulation test for chemical persistence in surface water. In this contribution, we first explore the pitfalls of the OECD 309 test, which include limited sensitivity, poor reproducibility, and lack of representativeness for biodegradation in the water body being simulated. This leads to the conclusion that most chemicals that pass the persistence screening assessment under REACH will be classified as persistent, which would seriously impact the ability of REACH to prioritize chemicals. We then explore several possible modifications to OECD 309, including adding larger quantities of sediment, focusing on the biodegradation kinetics during the first few days of the test, and spiking test chemicals at very low concentrations. We conclude that with these changes a sensitive, reproducible and much more representative test of persistence in aquatic systems is possible. Finally, we note that the dependence of the test result on the sediment : water ratio raises questions about lab-to-field extrapolation of half-lives and how the measured half-life can be compared with regulatory thresholds for persistence in water alone.

### 3.05.P-Th174 Integration-Optimization of Bioavailability Measurements and OECD 307 Test to Evaluate Persistence of Organic Pollutants During Incomplete Biodegradation Processes in Soil

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The incomplete biodegradation of organic pollutants in soil can rise the environmental risks if the transformation products are more toxic and mobile than the parent compounds. Alternately, the biodegradation products may remain as nonextractable residues, or as slowly desorbing but still extractable chemicals, which may be less risky than the parent, fast desorbing pollutants. The biodegradation process is itself influenced by the bioavailability of contaminants to the soil microbial populations, so bioavailability and risk assessment should be integrated and persistence studies combining these are limited. The significance of evaluating bioavailability during a biodegradation process has been examined in our previous studies on soil bioremediation, explaining that integrating bioavailability assessment gives a more realistic risk information than using the total contaminant concentrations only. Here, we studied the biodegradation by organic compounds in a prospective risk assessment scenario, with the OECD 307 simulation test incorporating bioavailability assessments. To assess bioavailability, the standardized ISO method (16751:2020) was used, which is based on a single-step desorption extraction with Tenax at 20 h. Two different organic pollutants (<sup>14</sup>C-pyrene and <sup>14</sup>C-carbamazepine (CBZ)) and different scenarios (soils with different content in organic matter, incubation periods and inoculation) were examined to follow the evolution of bioavailability of the parent compound and the transformation products from cometabolic reactions. The ISO method was adapted with CBZ, given its relatively low  $K_{ow}$  (= 2.7) by changing the soil/water/Tenax ratio. For both compounds, the bioavailability decreased and its phase-distribution changed during the transformation. At the end of the incubation, the fraction of transformation products present in Tenax were 48 % and 10 % of the initial concentration (4 mg/kg) for pyrene and carbamazepine, respectively. The products not trapped by Tenax but partitioned into the water were 3 % and 30 % of the initial pyrene and carbamazepine concentrations, respectively. The rest of the compounds remained as nonbioavailable residues.

### 3.05.P-Th175 Influence of Soil-Treatment on the Release of NER I

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Several guidelines define the experimental conduct, evaluation of data and, therefore, the criteria for the assessment of (physico-) chemical properties or toxicological and environmental behavior of chemicals. However, in environmental fate studies, the handling of nonextractable residues (NERs) remains vague and no standardized procedure is defined. Consequently, the risk assessment of NERs varies from potentially hazardous to “safe sink”. It is therefore of ubiquitous interest to be able to further characterize the nature of nonextractable residues and possibly subdivide them into further groups such as sequestered and entrapped residues (NER I), strongly bound residues (NER II) and biogenic residues (NER III). While NER II and NER III are of low or no environmental concern, NER I should be considered as potentially remobilizable.

To quantify the amount of NER I experimentally, several techniques are in discussion. These experiments aim to change the surface structure of the soil resulting in the release of entrapped molecules.

In this work, the influence of soil-treatment on the release of NER I using two different approaches is shown and discussed. Firstly, the feasibility of using EDTA to facilitate the release of NER I is presented. For this purpose, different types of soil (variation in organic matter, texture and cationic exchange capacity [CEC]) are treated with EDTA solution and the subsequent release of radiolabeled compounds is evaluated. Secondly, the influence of various silylation agents on different types of soil is shown. The correlation between bulkiness/rigidity of silylation agent as well as the influence of the CEC on the subsequent structural change and release of radiolabeled compounds is evaluated.

This contribution discusses feasibility, the conduct and the subsequent evaluation of postextraction soil treatments in order to differentiate between different types of nonextractable residues.

### **3.05.P-Th176 Integration of Quantification and Characterisation of Nonextractable Residues (NER) in Regulatory Persistence Assessment**

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Knowledge of the characteristics of nonextractable residues formed in degradation studies plays an important role in regulatory persistence assessment by providing further elucidation of the fate and behaviour of the chemicals in the environment. Characterisation of NER in reversible and irreversible bound fractions improves the confidence in interpretability of simulation degradation tests. There has been a long debate on the acceptability of the available NER characterisation methodologies. Therefore, a research project was initiated by UBA and carried out by a consortium of academia with the aim to test the practicability of different approaches for capturing NER in simulation degradation studies. Based on the extensive discussion and collaboration among regulators, academia, and industry, a stepwise approach for quantification and characterisation of NER has been developed. Step 1 of the approach specifies extraction methods for quantification of total NER and Step 2 describes NER characterisation by silylation or EDTA extraction to remobilisable and irreversibly bound fractions. Differentiation of NER types will improve interpretability of degradation study results and provides more robust information for assessment of persistence of substances. The updated ECHA Guidance on IR&CSA, Chapters R.11, R.7b and R.7c takes into account the outcome of these scientific advances in quantification and characterisation of NER and integrates this in the regulatory persistence assessment. This presentation will provide an overview of the scientific discussions leading to the integration of the quantification and characterisation of NERs in the regulatory persistence assessment.

### **3.05.P-Th178 The Performance of Aged Sorption Laboratory Studies: Considerations and Experience**

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Adsorption of chemicals to soil significantly influences their potential to move to groundwater and/or surface waters. Within the regulatory risk assessment procedure for pesticides, first tier assessments currently assume that pesticide sorption is instantaneous and fully reversible, and that the strength of adsorption is therefore constant with time. However, adsorption has frequently been observed to increase as the time of interaction between substances and soil also increases, i.e., 'aged sorption'.

As a result of these observations, it is becoming more common for experimental studies that demonstrate and quantify the increase in pesticide sorption with time to be submitted to regulatory authorities as part of the regulatory data package. The results of these studies are then used to revise estimates of predicted environmental concentrations in groundwater using FOCUS modelling. However, such studies are complex and difficult to standardise, and the results are often difficult to interpret.

A guidance document on the performance of aged sorption studies has been developed over many years and was originally approved and published on the EU Commission website in January 2021, with a revised version published in the following October. This guidance includes the design of the appropriate experimental protocol, standardisation of deriving the required parameters from the experimental data and how the parameters are subsequently used during exposure modelling. While the guidance is comprehensive, further knowledge and experience in the performance of aged sorption studies has been recently developed and will be communicated in this poster. Areas that will be explained more fully and need to be further considered when performing an aged sorption study include dealing with fast degrading substances, addressing the strict requirements regarding outliers and considering the value of preliminary investigations.

### **3.05.P-Th179 Degradation of Polyethylene by *Penicillium brevicompactum***

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The massive presence of plastics in the environment, and, specifically, in the marine environment, has become a topic of great concern in recent years.

Plastics have deleterious effects on marine fauna, and studies indicate that small plastics, dubbed microplastics, may have greater pervasiveness and impact.

Polyethylene (PE) is the most commonly found plastic in the environment, as it is also the most used polymer in our daily lives.

Biodegradation, the ability of some microorganisms to use a contaminant as source of nutrient, has been presented as a promising solution to reduce plastics already present in the environment and different microorganisms, including bacteria and fungi, have already been demonstrated to have the ability to biodegrade different microplastics. Within the Fungi kingdom, species of the *Penicillium* genus have been widely studied regarding this skill due to their large presence across the globe and their great ability to adapt and survive in different conditions.

*Penicillium brevicompactum* is commonly used to produce mycophenolic acid, an immuno-suppressant, and amenable to be grown and kept at industrial scale, and, as such, was chosen to assess its viability in the biodegradation of PE.

To test this hypothesis, three different experimental conditions were considered, with a one-month experiment, with four time points, one per week. We studied fungal growth in reduced medium with PE microplastics and fungal growth in a reduced medium without PE microplastics, and these two conditions were used to compare how fungal growth was influenced by the microplastics. The last condition was PE microplastics in a reduced medium, to see if any alteration present in the microplastics would be caused by the medium.

Our experiment showed that, in under 30 days, *P. brevicompactum* was able to reduce 50% of the microplastics present in the medium. In addition, it also showed that the fungus grew less when in contact with the microplastics. This may indicate that some nutrients required for fungal growth may be missing, so a possible next step would be to optimize the medium, with the objective of maximizing the removal of microplastics.

### 3.05.P-Th180 Biodegradation of a Range of Nonpolymeric and Polymeric Surfactants in Seawater

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Surfactants are a major class of chemicals commonly used in a wide range of domestic and industrial products. Despite efficient wastewater treatments, small fractions of surfactants may reach aquatic and eventually marine environments. Although many surfactants have been tested for ready biodegradability by standard freshwater OECD screening test guidelines, there is still a lack of systematic biodegradation data across various types of surfactants in the marine environment. In addition, little is known about seawater (SW) biodegradability of polymeric surfactants, such as the nonionic ethoxylated and/or propoxylated aliphatic alcohols. In this study, ultimate biodegradation of 18 surfactants representing different classes (including several polymeric alcohol ethoxylates), was determined in SW at 20°C by the OECD306 Closed Bottle test method. After 28 days of incubation, 12 surfactants reached 60% biodegradation and were considered to be readily biodegradable in SW. The results for the 6 additional surfactants indicated that the 60% threshold level may be reached by extended incubation time, or that reduced biodegradation could be associated with toxicity of the chemicals. All these 6 surfactants were biodegraded >20% after 28 days, indicative of primary biodegradation in SW. Polymeric ethoxylates with high numbers of ethylene oxide (EO) groups (40-50 EO groups) were more slowly biodegraded than polyethoxylates with 4 to 23 EO groups. A biodegradation experiment of the alcohol ethoxylate (AE) C12-EO9 (3 to 18 EO groups) was performed in a carousel system at 20°C with natural SW and a surfactant concentration of 500 µg/L. Rapid primary biodegradation was determined by targeted analyses (LC-MS) of the AE, with >99% primary biodegradation after 2 days of incubation. The surfactant depletion coincided with temporary formation of polyethylene glycols, suggesting that central fission is an important degradation step in SW. A primary biodegradation experiment in the carousel system with C12-EO9 was conducted in the presence of suspended particulate materials (SPMs; marine phytoplankton and clay particles, showing that the presence of SPMs did not hamper the primary biodegradation of the surfactant. Separation of fractions in 20 µm steel filters indicated some particle association of the surfactant.

### 3.05.P-Th181 Assessing Effects of Cationic Charge on the Biodegradation Profile of Cationic Guars Following OECD 302B with Concurrent GPC Analysis

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Hydroxypropyl guar hydroxypropyltrimonium chloride polymer (Common name: cationic guar, CAS number 71329-50-5) is a commonly used ingredient in personal care products, particularly hair care products, due to the cationic charge making the material a good thickener and conditioning agent. Additional benefits of using this material include a favorable human toxicity profile and a high ISO naturalness index score. However, highly charged materials may have the potential to be inhibitory to microbes and persistent in the environment. In this study, four cationic guars with various cationic degree of substitutions (cat DS) ranging from 0.08 – 0.22, including a low, medium, charge, and very high charge, were assessed following an extended (60 day) OECD Testing Guideline 302B (Zahn-Wellens/EMPA Test). In addition to biodegradation measurements, GPC analysis was performed at the following timepoints (days 0, 7, 14, 21, 28, 42, and 60) to measure changes in average molecular weight and molecular weight distribution of the polymers after such exposure. A general trend between increase in cationic charge and decrease in biodegradation percentage to day 28 was observed, with a threshold that a cationic guar could be classified as inherently biodegradable at approximately cat DS 0.20. Significant decreases in molecular weight and peak area were observed regardless of cat DS, however there was not a significant alteration in the pattern of reduction between differently charged guars. These results indicate that increasing cationic charge may negatively affect the biodegradation of cationic guars, as well as indicate the potential limits of OECD 302B with regards to charged materials. The additional GPC data provides evidence that it does not affect the method of degradation or the mineralization potential.

### 3.05.P-Th183 Fate of the Azole Fungicide Fluconazole in Sunlit Waters: Kinetics, Transformation Products, and Reaction Mechanisms

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The common antifungal pharmaceutical fluconazole is a persistent organic pollutant regularly found contaminating bodies of water around the world. Fluconazole can cause adverse health effects to at environmentally relevant concentrations and has been shown to inhibit the human cytochrome P450 system, which could slow metabolism of other xenobiotics and lead to synergistic toxic effects. Little is known about the environmental fate and transformations of fluconazole, although existing work on biodegradation and advanced oxidation processing indicates it is recalcitrant to many natural transformation pathways, often

requiring forcing conditions to degrade. Here we used the PhotoFate system to investigate the indirect photolysis of fluconazole in simulated sunlit freshwater environments. We estimate a half-life with respect to indirect photolysis in natural waterways ranging from around 5 to 25 days, with faster degradation observed with high concentrations of dissolved organic matter and/or nitrates. Transformation products have been identified and semiquantified using liquid chromatography coupled to (high resolution) mass spectrometry (LC-MS), with major products observed including multiple OH addition isomers, fragmentations of the bridging alkyl chain, and fragmentation of the aromatic ring. Tentative structures and reaction mechanisms to produce those structures are proposed based on exact mass analysis, retention time analysis, and previous observations of hydroxyl and carbonate radical reactivities in aqueous solutions. Notably, the electron-rich triazoleazole rings do not appear to be as prone to oxidation as we predicted, as stable OH addition products do not dominate the product profile indirect photolysis of fluconazole or 1,2,4-triazole, even when exposed to high OH radical concentrations by addition of hydrogen peroxide. We propose that either the hydroxyl radical causes slow fragmentation of theazole ring, resulting in volatile transformation products or OH addition products are formed, but they are very unstable and fragment in the electrospray ionization source, and are unlikely to be long-lived in the environment. Finally, the proposed transformation products will be tested for their binding energies to the cytochrome p450 isozyme CYP2C19 using a molecular dynamics approach to quantify the change in human cytochrome P450 enzyme inhibition following indirect photolysis of fluconazole.

### 3.05.P-Th184 Analysing the Biodegradation of Acesulfame in Lysates from Enriched Bacterial Cultures via Application of Enzyme Assays and Shotgun Metaproteomics

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The biodegradability of anthropogenic organic substances in wastewater treatment has been extensively studied, but information about the enzymes catalyzing the degradation reactions is scarce. In this context, we analyzed the enzymatic degradation of the artificial sweetener acesulfame (ACE), which is frequently detected in waters impacted by municipal wastewater. Biodegradation of ACE has already been proven and sulfamic acid (SA) was identified as quantitatively formed transformation product (TP). Genomes of ACE-degrading bacteria have been published lately, but the crucial proteins are still unknown and the degradation pathway has not yet been unequivocally clarified. To address this, we expanded the range of analytical methods used so far by combining i) enzyme assays with ii) protein fractionation and iii) proteomics analysis. We enriched ACE-degrading bacteria by incubating activated sludge in an artificial medium, using ACE as sole carbon source. A so far unknown *Chelatococcus* species and *Ensifer adhaerens* were identified as most abundant taxa after enrichment. Lysates were extracted next and fractionated via two-dimensional fast protein liquid chromatography. ACE was spiked to all fractions and enzyme activity was monitored by measuring the ACE degradation. Protein composition and abundances in all fractions were analysed using shotgun proteomics and label-free protein quantification. The protein fractionation succeeded in separating the enzyme(s) catalyzing the first degradation reaction of ACE from the enzyme(s) catalyzing the second reaction step sufficiently to detect an accumulation of the intermediate TP acetoacetamide-n-sulfonic acid (ANSA). ANSA has been previously identified in ACE-degrading cultures, but up till now was quantified only in trace amounts, leaving open the possibility of being solely the product of a sidechain reaction. The quantitative detection of ANSA observed here indicated that the initial step in the biodegradation of ACE is a hydrolytic ring cleavage at the sulfuric ester moiety, catalyzed by sulfatases or hydratases. Subsequently, ANSA must be degraded through cleavage of its amide bond catalyzed by amidases to form SA. A highly abundant metallo- $\beta$ -lactamase fold metallo-hydrolase was identified as most probable enzyme candidate for the catalytic cleavage of ACE by comparing protein abundances with the corresponding ACE degradation rates. Moreover, an amidase possibly catalyzing the degradation of ANSA was detected.

### 3.05.P-Th185 Microbial Degradation Potential of Emerging Contaminants by Isolated Bacterial Strains

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Emerging contaminants (CECs) are defined as unregulated or inadequately regulated chemicals. Many of them are used and released continuously into the environment, and may cause chronic toxicity, endocrine disruption in humans and aquatic wildlife. Bacterial degradation is an effective way in removing CECs either through metabolic or enzymatic pathways. High concentrations of CECs (musks; galaxolide (HHCB) and tonalide (AHTN)), UV-filters (Octocrylene (OC) and padimate-O (OD-PABA)) have been previously reported in sediments of a submarine canyon (Capbreton canyon). The bacterial strains belonging to the genus *Bacillus* sp. (S-1, S-2, S-3, S-5 and S-6) and the genus *Rhodococcus* sp. (S-4) have been then isolated from the marine sediments enriched with a single CEC and have been tested for their degradation potential against the CEC used for isolation. The aim of this study is to assess the biodegradation of CECs such musks (HHCB and AHTN); UV-Filters (OC and OD PABA); pharmaceuticals (ketoprofen and oxazepam) and pesticide (atrazine) by pure isolated strains related to different genus. The degradation potentials of these compounds were also discussed considering to chemical structure of CECs, phylogeny, and resistance of the strains.

The strains were cultivated in MM<sub>20</sub> medium and were spiked with a single CEC at 1 mg L<sup>-1</sup> for 24 hours incubation for *Bacillus* sp. and 48 hours for *Rhodococcus* sp. HHCB, AHTN, OD PABA, OC and Atrazine. The results show that some strains have high degradation potentials against CECs (S-3, S-4 and S-5 strains) whereas others are only able to degrade OC (S-1 and S-2). S-6 was not able to degrade any CEC. For those with high degradation potentials, 5 CECs (HHCB, AHTN, OD-PABA, OC, and

ketoprofen) were degraded with different potentials meaning that the degradation extent is CEC-dependent. The results also reveal that atrazine and oxazepam are highly persistent CECs as no degradation by any strain was observed. Moreover, the resistance of the selected two strains belonging to *Rhodococcus* sp.(S-4) and *Bacillus* sp.(S-5) was investigated by exposing HHCB, AHTN, OC, OD-PABA, and ketoprofen up to 500 mg L<sup>-1</sup>. The results showed no toxicity effect for musks and UV-Filters. However, the growth was inhibited when more than 30 and 60 mg L<sup>-1</sup> of ketoprofen of S-4 and S-5 were used, respectively. Overall, the experimental results demonstrate that degradation ability of the strains is not linked to toxicity or resistance to CEC.

### **3.05.P-Th186 Determining Marine Biodegradation Kinetics of Chemical Mixtures Discharged from Offshore Oil Platforms – Mixture Toxicity Inhibited Biodegradation at Low Dilutions**

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Enormous volumes of produced water carrying complex chemical mixtures are discharged to the marine environment from offshore oil platforms. Environmental Impact Assessments (EIAs) focus generally on the (eco)toxicological effects of these discharges in the near field, but it is equally important to avoid large discharges of chemicals that are persistent which can build up in the environment. This asks for new approaches to determine marine biodegradation kinetics of discharged chemicals at low environmentally relevant concentrations.

This study aimed to (1) determine biodegradation kinetics of chemicals in produced water (discharged chemical mixture) diluted in seawater (inoculum microorganism at discharge) and (2) determine how the dilution factor affects biodegradation kinetics. Produced water and seawater samples were received from an offshore oil platform in the North Sea and biodegradation experiments started within 32 hours. Parallel experiments were conducted with produced water diluted in seawater by factors of 20, 60, and 200, and with a gasoline calibration standard (23 reference substances) spiked in seawater. The test systems were prepared in 20 mL gastight autosampler vials and incubated up to 60 days at ambient seawater temperature (10°C). At predetermined time points, automated Solid Phase Microextraction coupled to GC-MS was applied directly on test systems to determine constituent specific biodegradation based on biotic to abiotic peak area ratios (i.e., primary biodegradation). The gasoline calibration standard was used to identify constituents in the produced water. Biodegradation kinetics of 15 reference substances were determined at all tested dilutions and generally showed faster biodegradation with increasing dilution factor. Toxicity tests of the produced water using marine algae and luminescent bacteria yielded EC<sub>50</sub> values in the same range as the highest concentration in the biodegradation experiments, which suggests mixture toxicity inhibiting microbial degraders at low dilutions. These results emphasize the linkage between mixture toxicity and biodegradation kinetics and underline the importance of conducting biodegradation tests at low environmentally relevant concentrations. Biodegradation kinetic data are highly valuable for assessing the environmental risk of discharged chemicals and can focus management efforts on persistent and slowly degradable chemicals where substitution or treatment before discharge might be necessary.

### **3.05.P-Th187 Emerging Polychlorinated Biphenyl Dechlorination Congener Profiles in Five Environmental Compartments In and Near the Mohawk Community at Akwesasne**

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The industrial area west of the St. Regis Mohawk Tribe (SRMT) in Akwesasne, NY, USA, along the St. Lawrence River, has been heavily impacted by polychlorinated biphenyl (PCB) contamination since at least the 1950s, with three Superfund PCB sites located within 18 km of the reservation's western border. Sampling of five environmental compartments in the area (air, ground water, soils, sediments, tree bark) since 2020, and analysis of 209 PCB congeners, show evidence of PCB dechlorination: In all but the soil samples neurotoxic dechlorination end-point congener 2,2'-dichlorobiphenyl (PCB-4) dominates the congener profile in at least one sample, or is among the four most abundant congeners in some soil samples. The neurotoxic activity of 2,2'-dichlorobiphenyl is inhibition of dopamine synthesis. While this congener is not considered to be bioaccumulative ( $\log K_{ow} < 5$ ), its production in the environment is apparently continuous because of the results from air samples. Intermediate dechlorination PCB congeners include 2,3,2',5'-tetrachlorobiphenyl (TetraCB), 2,4,2',4'-TetraCB, 2,3,2'3'-TetraCB, which are also found in many Akwesasne samples. Dominance of PCB congener profiles by 2,2'-dichlorobiphenyl is not found in Aroclor products, and it is not more than 0.32% of the Aroclor 1248 used at the Superfund sites, so there is no significant historic source. Among these five compartments, one (tree bark) comes from near drainage of the western Superfund site, while the others were from the eastern Superfund site where total PCB contamination amounts are high. The effect of dechlorination on these five environmental compartments at and near Akwesasne is apparently active throughout the area. We hypothesize that the observed dechlorination process involves organisms in contaminated ground water or landfill leachate, with some of the 2,2'-dichlorobiphenyl volatilizing to the atmosphere, and then is found in what is considered to be a passive air sampler – tree bark. This is a recent discovery because PCB congener analysis in environmental samples has previously been limited at the site. PCB dechlorination was not suspected in congener-specific PCB air analysis from this site in 1993 because no evidence was observed in results at the time. The recent appearance of 2,2'-dichlorobiphenyl to these five environmental compartments presents a previously unrecognized health compromise to residents in and near Akwesasne.

### **3.06 Climate Change in Arctic and Antarctica and Its Effect on Legacy and Emerging Micropollutants in Abiotic and Biotic Environmental Compartments**



### 3.06.P-Tu152 Pharmaceuticals and Personal Care Products in the Kongsfjorden Ecosystem (Svalbard, Norway)

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Climate warming (CC) is occurring faster and with greater magnitude in the Arctic compared to other parts of the world, causing significant effects on the environment (faster sea ice retreat, melting glaciers, thawing permafrost, extreme weather events and alterations of atmospheric and oceanic circulation and biogeochemical cycles). On one side, these processes alter the contaminant fate, transport and distribution into ecosystems, on the other hand, the Arctic warming has favoured the development of anthropogenic activities (e.g., fisheries, tourism and resource extraction), which are additive local sources of pollutants, also considering the low technologies of wastewater treatment plants currently used.

Among chemicals of emerging Arctic concern, pharmaceuticals and personal care products (PPCPs) have recently attracted increased attention, due to their bioactive nature and potential effects on natural microbial communities and, thus, on the structure and functioning of the ecosystem. PPCPs are considered “pseudo-persistent contaminants” because they are daily-use products continuously released into the environment. Depending on their chemical-physical properties, some PPCPs have a relatively high lifetime and can be long-range transported from mid-latitudes.

The current knowledge on the distribution of PPCPs in the Arctic ecosystems is still limited, even though the global consumption of many PPCPs has increased since 2020 due to the COVID-19 pandemic.

In this context, the present work aims at evaluating the occurrence, distribution, and sources of selected PPCPs in the Kongsfjorden marine ecosystem (KF, Svalbard Norway). Seawater and sediment samples were collected in a grid of points along KF, from the Ny- Alesund (NA) Village, where research scientific bases are settled, to the main glaciers.

Target PPCPs were detected in seawater and sediment samples, suggesting their ubiquitous occurrence. In particular, concentrations of pharmaceuticals, caffeine and insect repellents ranged between n.d. and 100 ng/L in seawater samples. PPCPs occurred also in sediment samples with a concentration range between n.d. and 200 ng/g d.w. Higher concentrations were found in the sampling points close to the NA village, suggesting an important role of local sources of contamination, even if a contribution from atmospheric and sea current transport cannot be excluded.

### 3.06.P-Tu153 Are Ingredients of Personal Care Products Likely to Undergo Long Range Transport to Remote Regions?

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Personal Care Products (PCPs) contain contaminants of emerging concern (CECs). Whereas their occurrence in environmental matrices in polar regions has been reported, little is still known about their behaviour under cold environmental conditions. A selected group of PCPs was studied to understand their potential for atmospheric and oceanic transport to remote regions and their environmental fate in cold environments. In this study, we (I) estimated the key chemical properties of the PCPs and their temperature dependence, (II) calculated the equilibrium phase distribution in the atmosphere and the dominant deposition processes at temperatures above and below freezing, and (III) simulated their potential for long-range transport (LRTP). The chemical properties were estimated using a variety of methods and databases. We predicted the atmospheric behaviour by superimposing the predicted properties onto chemical space plots that display the equilibrium phase distribution in the atmosphere and the dominant deposition mechanisms at 25° and -6.8°C. The LRTP of the selected PCPs, when emitted to air, water, and soil, was estimated using the emissions fractions approach as implemented in a modified version of the OECD Screening Tool. Most of the selected PCPs, especially the fragrance materials, are expected to be partitioning mostly into the atmospheric gas phase but are also sufficiently water soluble to be subject to precipitation scavenging. Especially at lower atmospheric temperatures (-6.8°C), scavenging with snow is predicted to be the dominant atmospheric deposition process for all investigated PCPs. The dispersal is highest if the chemical is emitted into the air, while, for most PCPs, transfer, and accumulation in surface media of remote regions are more pronounced when emitted into water. The relatively low LRTP estimated for the selected PCPs is at odds with the detection of those PCPs in surface media of remote polar regions. Future work should seek to reconcile this discrepancy. Potential hypotheses include: (i) the presence of PCPs in remote regions is a result of local sources and/or (ii) the degradation half-lives of the PCPs are greatly underestimated with the prediction methods used in this work.

### 3.06.P-Tu160 Contaminants of Emerging Concern in the Arctic Fjord Kongsfjorden: from Glaciers to Marine Ecosystem

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Climate warming, which is occurring most rapidly in the Arctic, is leading to strong alterations in atmospheric and oceanic circulation and hydro-biogeochemical cycles as well as has favoured a major human presence in this area. All these aspects affect directly or indirectly fate, transport and distribution of contaminants within the Arctic environment. Due to the presence of international scientific stations, Kongsfjorden (KF- Svalbard, Norway) has been extensively investigated for many years. This

small Arctic fjord is directly influenced by the inflow of warm Atlantic water and various land and tidal glaciers. The anthropic impact (cruise tourism, Ny-Ålesund research stations etc.) is also increasing in this area. It is, therefore, a highly sensitive marine system to climate change (CC) and constitutes a representative environmental monitoring area to evaluate the impacts induced by CC on new emission sources, remobilization, transport and circulation of contaminants.

Among them, the chemicals of emerging Arctic concern (CEACs) have gained considerable attention for their potential toxic effects on humans and wildlife. Studies on the environmental dynamics of CEACs in the Arctic ecosystems, as well as on the better-known POPs (Persistent Organic Pollutants), are of strategic relevance because the impact of CC on the spread of contamination in the Arctic can provide early warning signals for similar repercussions on a global scale.

In this context, the present study aims at investigating the occurrence and distribution of selected CEACs and POPs, such as old and new-generation pesticides, bisphenol A and nonylphenols (NPs) in the KF ecosystem. The study area includes sampling sites strongly affected by tidal and land glacier runoff and sites locally impacted by human settlements. The Bayelva catchment including the two land terminating glaciers, and their periglacial/proglacial area until the river mouth, was also investigated both before and during the melting period. Contaminants were determined in floor sediment and seawater of KF, as well as in snow and drainage waters by high sensitive chromatographic determinations.

Target compounds were found in all environmental samples analysed, suggesting their ubiquitous presence in the Arctic ecosystem. Higher concentrations were found for NPs in all matrices. The overall results indicate that both local sources and glacier runoff, can contribute to the contaminant load in the KF marine ecosystem.

### **3.06.T-01 Stable Isotope Values (d13C, d15N) Reveal Long-Term Bioaccumulation of Perfluoroalkyl Substances (PFASs) in Icelandic Seabirds**

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Exposure to the environmentally persistent perfluoroalkyl substances (PFASs) has been linked to harmful health impacts in humans and fauna. The ability of PFASs to biomagnify through food webs make high trophic level consumers particularly vulnerable to exposure. Although PFASs have produced on an industrial scale since the 1950s, our understanding of their sourcing, exposure and bioaccumulation in the environment is limited, especially for Arctic consumers.

This study seeks to fill this knowledge gap by characterizing PFASs patterns and levels in two congeneric seabird species in Iceland, the true Arctic Brünnich's guillemot (*Uria lomvia*) and the temperate Common guillemot (*Uria aalge*). Iceland is a key region for understanding anthropogenic impacts in Arctic regions because it hosts both temperate and arctic species, and the waters around Iceland are fed by warm Atlantic currents introducing contaminants into the Arctic Ocean, but also from cold Arctic Overflow. In addition, global warming is causing a decline in Arctic species on the island.

By combing analyses of commonly observed perfluorooctane sulfonic acids (ΣPFOS, linear and its isomers), perfluoroalkyl carboxylic acids (PFCAs), and bulk stable isotope of blood plasma samples retrieved from five geographically distinct bird colonies during their breeding season in 2018, we test species differences and whether PFASs levels in blood plasma reflect current or past season diets. Linear PFOS is particularly interesting because of its high bioaccumulative potential and wide occurrence in Arctic wildlife.

Across all birds, we observed a generic PFCA profile with high proportion of odd and long carbon-chain length homologues, i.e. ,PFNA, PFUnDA and PFTrA. We found significantly higher PFCA concentrations in *U. aalge* than *U. lomvia*. Since the correlation between PFCA exposure and recent diets (as inferred from the stable isotopes) is very weak, we posit that the birds incorporated most PFASs prior to the breeding season. ΣPFOS concentrations were generally not linked to species but rather to the geographical location of the breeding colonies: Birds sampled in southern Iceland almost had twice the exposure than birds from northern Iceland. In summary, our PFAS results suggest that the high exposure levels of *U. aalge* are linked to winter migration in proximity to densely populated regions. More studies are needed to characterize the linkages between PFAS concentrations and the birds' wintering habitats

### **3.06.T-02 Organophosphorus Esters in a Firn Core from Austfonna, Svalbard**

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In spring 2015 we drilled a 13.2 m deep firn core representing dates mid-2001 to early-2015 from Austfonna, the largest ice cap on Svalbard. The core was separated into 18 samples for analysis of 13 organophosphorus esters (OPEs). Six OPEs were detected in at least 18 samples: The two OPEs with highest flux in most samples were tris(2-chloroisopropyl) phosphate (TCIPP) and tri-*n*-butyl phosphate (TNBP), each reaching maximum values of nearly 3500 pg cm<sup>-2</sup> yr<sup>-1</sup> in the surface sample. Correlation of these values among all 18 samples was 0.775, significance <<0.05, suggesting a similar environmental source over time. These two OPEs have different uses, suggesting that they have been used consistently at the source sites. These maximum flux values are nearly four times greater than maximum brominated flame retardant (hexabromocyclododecane, 910 pg cm<sup>-2</sup> yr<sup>-1</sup>) or for a current-use pesticide (chlorpyrifos, 809 pg cm<sup>-2</sup> yr<sup>-1</sup>) both measured in the Svalbard ice core at Høltedahlfonna (2005) showing both that OPEs have greater input and high amounts of transport and deposition to Austfonna. The second-most abundant OPEs included tris(2-chloroethyl) phosphate (TCEP) and triphenyl phosphate (TPHP), but not varying consistently with a correlation of 0.325

and significance 0.19, showing that there is no relationship with these OPEs and apparent different sources over time. Two other OPEs detected are tricresyl phosphate (TMPP) and tris(2-ethylhexyl) phosphate (TEHP). Both are detected in all samples but both with fluxes about 100 times less than TCIPP and TNBP, typically not greater than  $30 \text{ pg cm}^{-2} \text{ yr}^{-1}$ . The fraction of each of these six OPEs when summed showed high variability in principal components analysis when the input values were comparatively low. Air particle samples collected in Longyearbyen, a town 230 km southwest from the Austfonna drill site, showed maximum OPE values for TNBP and 2-ethylhexyl-diphenyl phosphate (EHDPP), the latter not detected in any sample at Austfonna. At Longyearbyen, the TCIPP amount was about three times greater than TCEP, similar to the ratio at Austfonna. TCIPP, the most concentrated at Austfonna, was fourth most-concentrated at Longyearbyen. The non-chlorinated OPEs comprised ~75% of all OPEs in Longyearbyen air in contrast to ~50% in Austfonna firm. These contrasts show that Longyearbyen is not a likely OPE source to Austfonna, if transport and deposition are equal among OPEs.

### 3.06.T-03 First Detailed Assessment of Organic Contaminants in Killer Whales Across the North Atlantic Ocean and the Influence of Diet Composition

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Recent studies have highlighted the critical threat posed by ongoing, high concentrations of persistent organic pollutants (POPs) in cetaceans and particularly in killer whales due to their high trophic position and limited biotransformation and elimination capacity. Although tissue concentrations of legacy POPs are reasonably well documented for Northeast Pacific killer whales, considerably less is known for North Atlantic (NA) conspecifics and for new or emerging POPs. Although POP concentrations are influenced by feeding habits, precise estimates of killer whale diet composition, especially in remote areas of the NA Ocean, were not available until recently. Here, we use our newly developed quantitative fatty acid signature analysis (QFASA) approach to estimate the diet composition of ~200 NA killer whales from 2008 to 2022. Then, we quantify POP concentrations in these same individuals and assess the relationship between diet composition and POP concentrations. Diet estimates showed that killer whales mainly consume other whales in the western NA, seals in the mid-NA, and fish in the eastern NA. Nonetheless, diet estimates also varied widely among individuals within most regions. Concentrations of PCBs ranged from a high of ~ 100 mg/kg lw in the western NA, to about 50 mg/kg in the mid NA, to safer levels in the eastern NA. Generally similar patterns were observed for DDTs and CHLs. Based on a threshold for health risk at 10 mg/kg lw and high risk at 100 mg/kg lw, killer whales in the western NA face high risks of health effects due to PCBs alone. The diet of killer whales in the NA explained up to 60% of the variation in PCB concentrations. These results indicate how critical this feeding variation among NA killer whale groups is for their resulting contaminant loads. Nonetheless, intrapopulation differences were also found and detailed analysis including other biological variables (e.g., sex) will serve to further clarify the drivers of these substantial differences in contaminant concentrations and associated health risks.

### 3.06.T-04 Small Microplastics, and Microlitter Components in Superficial Water and Sediments of Krossfjorden, Svalbard Archipelago

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Small microplastics (SMPs, <100 µm), part of the microlitter, can reach high latitudes through long-range transport, but they can also be released locally by human settlements and commercial/tourist activities. In the same way, other microlitter components (MLCs), i.e., plastic additives, plasticizers, and natural and nonplastic synthetic fibres, can be carried from mid- to high latitudes or released into the environment. Microplastics > 100 µm were studied at high latitudes, while small microplastics, which can easily enter the food web due to the ingestion by invertebrates, have been overlooked. So far, the occurrence and distribution of plastic additives and plasticizers have never been investigated. Additives and plasticizers are generally included in plastic objects and can therefore be proxies for the presence of specific plastic polymers.

In the summer of 2022, samples of surface seawater and marine sediments were collected in different sites of the Krossfjorden in the Svalbard Archipelago. Sampling activities were performed on board of MS-Teisten (Kings Bay vessel), using Niskin bottles and Van Veen Grabs. Field blanks were collected as well. At the “Dirigibile Italia” base, sediment samples were divided into decontaminated glass jars, while seawater samples were filtered on glass fibre filters. All the samples were stored at 4°C before being shipped to Italy. Laboratory blanks were collected to check the contamination. At the CNR-ISP in Venice, samples were pretreated to extract simultaneously small microplastics and the microlitter components, employing an oleo-extraction procedure which does not further degrade plastic particles and MCLs. Reagent and procedural blanks, together with those collected at Svalbard's, were pretreated and analyzed as samples. The analytical phase was performed with the Micro-Fourier-transform Infrared spectroscopy.

The results of this study provide us with information (i) on the identification and quantification, via microscopic counting, of SMPs and MLCs in surface seawater and sediments samples, (ii) on the sources, transport and fate of these pollutants. This study will be a starting point for increasing knowledge about plastic additives and plasticizers in the Arctic environment.

### **3.06.P Climate Change in Arctic and Antarctica and Its Effect on Legacy and Emerging Micropollutants in Abiotic and Biotic Environmental Compartments**

#### **3.06.P-Tu155 Quantification of Water Discharge From the Bayelva Glacial River and Water-Driven Pollutant and Microbe Fluxes into Kongsfjorden (Western Svalbard-Norway)**

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Climate change heavily affects Arctic hydrologic dynamics, generating significant environmental modifications and potentially leading to climatic feedback and further warming amplification. In formerly glacierized watersheds, hydrologic processes are evolving, with new storage mechanisms and distribution of water resources, as more persistent rivers and developed groundwater systems. However, water balance still cannot be considered good enough to assess the individual components of the hydrological cycle with sufficient accuracy, including groundwater, which can play a key role for ecosystems and human communities in Arctic regions. Changes in river flows will alter fluxes of freshwater, sediments, and nutrients with implications for biodiversity in both freshwater and marine ecosystems. The rapid glacier melting affects weathering processes, resulting in the mobilization-transport of different inorganic and organic molecules (i.e., pollutants), microorganisms stored for a long time, and turbid meltwaters. Thanks to its geographical characteristics, the retreating glaciers, the research stations and infrastructures, and the studies carried out in the past and present, the Bayelva catchment near Ny-Ålesund (Western Svalbard-Norway) is an ideal site for surveys aimed at increasing knowledge on hydrology dynamics and associated effects, in the continuum from glaciers to the fjord.

In this framework, within the ICEtoFLUX project (MUR/PRA2021 project-0027) a field campaign was conducted both in the spring and summer of 2022 in the Bayelva River catchment, from its glaciers and periglacial/proglacial systems up to the Kongsfjorden zone significantly affected by the river. The activities were aimed to quantify hydrologic processes and related transport of inorganic-organic chemical compounds (counting pollutants) and microbial biomass and activities. Chemical results from snow, drainage water, and seawater samples are presented here, together with discharge measurements. The first results suggest that, in general, electrical conductivity and total suspended solids increase from the glacier to the Bayelva monitoring station, which is located less than 1 km far from the coast. Seasonal evolution of physical-chemical features was also observed. The marine samples collected near Bayelva mouth show the influence of freshwater input and changes in turbidity. First evidence on organic pollutant and microbe transport is also discussed.

#### **3.06.P-Tu156 Using Passive Air Samplers to Sniff Out Local Sources of Persistent Organic Pollutants (POPs) at Remote Sites**

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Local sources of POPs and related chemicals at remote sampling locations (e.g., land-based stations, polar stations, ship-based sampling) can lead to challenges for the integrity of air monitoring studies. Potential emissions associated with current or past human activity or infrastructure can generate a “halo” of elevated concentrations that will depend on the magnitude of the emission. Passive air samplers are proposed as a simple and effective tool for identifying local sources of POPs.

A mapping study was conducted at the Alert, high Arctic air monitoring station to assess homogeneity and potential hot spots / local sources of selected POPs in air. Samples were deployed using polyurethane foam disk passive air samplers (PUF-PAS) at approximately 10 sites during October 2004 to March 2005 (Period 1) and March 2005 to October 2005 (Period 2) with replicate samples collected at some sites. Period 1 samples were analyzed for organochlorine pesticides (OCPs), including (hexachlorocyclohexanes (HCHs; a-, b-, g- and d- isomers); heptachlor, heptachlor epoxide (HepX), aldrin, dieldrin; sum of chlordanes i.e., trans-chlordane (TC), cis-chlordane (CC), trans-nonachlor (TN); o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT; and sum of endosulfans i.e., endosulfan I, endosulfan II, and endosulfan sulfate. Period 2 samples were analyzed for polychlorinated biphenyls (PCBs) (sum of 56 congeners) and polybromodiphenyl ethers (PBDEs) (sum of 17 congeners).

Results show that none of the OCPs or the PCBs exhibited “halos” of concentrations that would indicate a significant local source. However, PBDEs were elevated at a site in the southwest corner of the base, where newly installed infrastructure was suspected of being the local source. The “halo” of PBDEs was very localized and diminished beyond approximately one hundred meters and only impacted a few nearby adjacent sites. PUF-PAS are recommended as a simple approach for investigating potential local sources of gas- and particle-associated POPs that may impact monitoring at remote sampling platforms.

### 3.06.P-Tu157 Small Microplastics (<100 µm) and Microlitter Components in Antarctic Snow

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Despite their versatility and long-lasting quality, plastics can degrade, producing particles with different shapes and sizes; those particles < 5 mm are called microplastics and have been observed in seawater, sediments, and soil. There is scientific evidence that organisms ingested microplastics (MPs); in particular, invertebrates at the bottom of the food web can ingest MPs < 100 µm (small microplastics, SMPs), according to the size of their mouthparts. Once entering the trophic web, SMPs may be bioaccumulated and even biomagnified, posing a threat to human beings as well. By their definition, plastics may contain plastic additives, i.e., plasticizers, antioxidation agents, lubricants, antistatic agents, pigments, etc. When plastics are degraded into the environment, plastic additives may be exposed in different shapes and released into the environment, becoming part of the microlitter together with natural and non-plastic synthetic fibers (microlitter components, MLCs). While MPs > 100 µm were investigated in the mountain and polar snow, SMPs and especially MLCs are still overlooked.

In this study, surface snow was sampled at different sites in Antarctica. Field blanks were collected during the sampling, and snow samples were stored in decontaminated glass bottles; after sampling, they were stored at 4 °C before being shipped to CNR-ISP, Venice, Italy. Quality assurance and quality control (QA/QC) were strict to check the contamination at any step, from sampling to analysis. Once in Italy, all the samples were pretreated (via oleo-extraction previously developed at CNR-ISP, Venice); field blanks, reagent and procedural blanks were pretreated as samples. All the operations were performed in a plastic-free ISO 7 clean room. Oleo-extractions were then filtered on aluminum oxide filters (porosity 0.2 µm, 47 mm) and purified. Filters were dried in the clean room at room temperature in decontaminated Petri dishes covered with decontaminated aluminum foil, and then they were analyzed via Micro-FTIR. Identification and quantification via microscopic counting of SMPs and MLCs were performed simultaneously in semiautomated mode; only spectra with a similarity match percentage equal to or greater than 65% were regarded as identified and thus were quantified. Preliminary data are presented regarding the abundance, size distribution, and weight of SMPs and MLCs.

### 3.06.P-Tu158 Small Microplastics (<100 µm), Plastic Additives, and Other Microlitter Components in Snow from Different Glaciers in Svalbard Islands

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Most of the recent studies have focused on microplastics (MPs) presence and transport in aquatic and terrestrial ecosystem, while airborne MPs are very limited investigated. The smallest and lighter microplastics (e.g., SMPs small microplastics < 100 µm) can easily be transported over long distances to receptor and pristine regions, such as Arctic. Besides the presence of SMPs, there is a fundamental research gap in understanding long-range transport of microlitter components (MLCs), such as plastic additives, to the remote areas via atmospheric deposition. Further, there is a significant lack of standardized methods on these contaminants regarding their sampling, quality assurance and quality control (QA/QC) protocols, the pretreatment, quantification, and chemical identification. In this research different snow samples from snow pits were collected from April 2021 at the Gruebadet site located in the proximity of the Ny-Ålesund settlement (78.918 N, 11.895 E) and at the summit of different glaciers: the Midtre Lovénbreen (MDL; 78.871N, 11.984E), the Austre Brøggerbreen (ABG; 78.872N, 11.915E) and the Høltedahlfonna (HDL; 79.140N, 13.393E). Specifically, the annual snowpack was sampled separating the upper layer (superficial fresh snow deposition) and the lower layer to the ground. A detailed QA/QC was designed to avoid/minimize potential plastic contamination of samples during all the operations until the analysis. All samples were pre-treated using an oleo extraction-method previously development from CNR-ISP. The quantification and chemical identification of SMPs and MLCs were carried out via Micro-FTIR. The superficial layers were evaluated to study the temporal variability of SMPs and MLCs; while the bulk layers were investigated for the spatial distribution, since these layers are more conservative. Weight, polymer typology and size of SMPs and MLCs were calculated for each glacier. Nylon was one of the most abundant typologies analyzed as SMPs; while rayon and different plastic additives (e.g., vulcanizing agents, cosmetic additives...) were identified. The total load (µg SMPs/m<sup>2</sup> /d) between Gruebadet site, near Ny Alesund scientific urban settlement, and the summit of the glaciers, confirmed that SMPs could be influenced by long-range transport even in remote sites. Further, the presence of MLCs, at the summit of the glaciers confirming their role as SMPs proxy in environmental matrices and a potential threat for biota in polar ecosystems.

### 3.06.P-Tu159 Personal Care Products in Northern Greenland: First Results in Snow Samples from Station Nord

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Human activities have both direct and indirect impacts on the delicate arctic environment. For years, in the Arctic the attention was mainly focused on legacy contaminants, still, the Arctic Monitoring Assessment Program recently stressed the importance of monitoring a new set of compounds defined as Chemicals of Emerging Arctic Concern (CEACs). These chemicals are present as ingredients in Personal Care Products (PCPs) such as shampoos, perfumes, detergents, and sunscreens. A limited number of studies show the occurrence of PCPs in environmental matrices in polar regions and, to date, the general monitoring of CEACs in Greenland is limited. Most of the available studies focused on sewage sludge, freshwater sediments, air, and marine biota. To the best of our knowledge, no studies have yet investigated the occurrence of PCPs in the snow of Greenland.

In this study, we investigate the occurrence and the temporal trends of a selected group of PCPs in snow: seven fragrance materials (Amyl Salicylate 1 and 2, Oranger Crystals, Hexyl Salicylate, Peonile, Ambrofix, and Benzyl Salicylate), four UV-filters (EHMC, BP3, EHS, and Octocrylene), BHT, and BPA. The snow samples were collected once every two weeks in the area surrounding the Villum Research Station (VRS) in Station Nord (81°36' N, 16°40' W; 30 m a.s.l.), between December 2018 and June 2019. All the samples were prepared in a stainless clean-room for organics (class 10000) and analysed with GC-MS/MS at the Ca' Foscari University laboratories in Venice.

The preliminary results show the presence of most of the selected chemicals. Particularly, the UV-filters and BPA are the most occurring compounds. The cumulative concentrations of PCPs increase in Spring, and they reach the maximum in May. The VRS operates all year round and it can host up to fourteen scientists. The period with high cumulative concentrations of PCPs coincides with the all-day long-lasting sunlight season in the Arctic. During these months the need for sunscreen becomes important for people operating in the area, and this can explain the increasing concentrations of UV-filters. However, concentrations of the selected PCPs are found throughout the whole sampling period so we cannot exclude they also underwent regional or long-range transport.

### **3.06.P-Tu161 Assessment of Polycyclic Aromatic Hydrocarbons and Polychlorobiphenyls in Arctic Marine Sediments (Svalbard Islands, Norway)**

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The Arctic is characterized by extreme seasonal light variations, low temperatures, short growing seasons, and limited availability of nutrients, making this ecosystem one of the least productive and species-poor in the world. For these reasons, this cold area is ecologically sensitive to global and local impacts, including climate change (CC) and pollution.

Anthropogenic contaminants can be transported from lower latitudes into Arctic regions through sea and atmospheric currents, rivers, and migratory animals. Global warming is affecting atmospheric and oceanic circulation processes, leading to sea ice reduction, increased seawater temperatures, and a higher influx of Atlantic water masses, altering, in turn, the contaminant transport, distribution and fate in the Arctic. In addition, the increase in glacier melting and permafrost thawing can remobilize pollutants previously trapped, representing a secondary source of local pollution. An increase in “old” contaminants in Arctic seawaters, including some persistent organic pollutants (POPs), banned many years ago, has been found, triggering a possible mechanism to promote the transformation of the Polar Regions from sink to a source of pollutants. The development of fisheries, cruise tourism, and resource extraction, favoured by the increasing inflow of warmer Atlantic and Pacific waters and reduced sea-ice cover, has increased economic benefit for the coastal Arctic communities as well as the anthropogenic contamination on a regional scale ([www.amap.no](http://www.amap.no)).

The present study investigates the occurrence of polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) in marine sediments collected in Kongsfjord (Svalbard, Norway) during the summer of 2018 and 2022. The contribution of different local contamination sources as well as the risk assessment for the marine environment were also evaluated.

The results indicate that the combination of long-range transport, glaciers melting, as well as local anthropic activities, contributes to the contaminant load in the Kongsfjorden ecosystem. A comparison between the concentrations of target compounds measured in Kongsfjorden sediment and empirical and mechanistic indices derived from sediment quality guidelines, suggests that the occurrence of PAHs and PCBs in the study area does not currently pose a risk for this Arctic ecosystem, but further investigation on the spread of hazardous contaminants and their effects on these fragile environments is needed.

### **3.06.P-Tu162 Diffusive Fluxes of Persistent Organic Pollutants Between Arctic Atmosphere, Surface Waters, and Sediments**

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Arctic communities are disproportionately exposed to persistent organic pollutants from sources including global atmospheric transport and formerly used defense sites (FUDS). The effects of global climate change and increasing development in the Arctic have the potential to exacerbate this problem. Yupik residents of Sivuqaq (also known as St Lawrence Island), Alaska are one such community with documented exposures to pollutants from FUDS, and traditional foods like blubber and rendered oils of marine mammals. Troutman Lake, adjacent to the Yupik village of Gambell, Alaska, was used as a disposal site during the decommission of the adjacent FUDS, leading to community concern about exposure to legacy military pollution. In collaboration with a local community group, this study utilized passive sampling devices deployed at eight locations in Troutman Lake. Air, water and sediment pore-water deployed samplers were analyzed for 41 unsubstituted and 22 alkylated polycyclic aromatic hydrocarbons (PAHs), 43 brominated and organophosphate flame retardants and 52 polychlorinated biphenyls (PCBs). Sum PAH

concentrations ranged from 20 – 55 ng/m<sup>3</sup> in air, 1.4 – 3.2 ng/L in surface water and 0.46 – 1.4 ng/L in sediment pore-water. Naphthalene was the most abundant PAH in the air and water, constituting more than 70% of total PAHs. In the sediment pore-water, PAHs were primarily three ring compounds, in particular, phenanthrene and sulfur heterocycle dibenzothiophene. The majority of PAHs were in deposition from the overlying atmosphere into Troutman Lake. Brominated diphenyl ether 47 was detected in all surface water samplers (0.0018 – 0.0156 ng/L). Organophosphate flame retardant, triphenyl phosphate was detected in all environmental compartments at concentrations less than 0.004 ng/m<sup>3</sup>, 0.07 ng/L and 0.07 ng/L in air, water and sediment porewater, respectively. Of particular interest, we measured higher vapor phase concentrations of tris(2-chloroethyl) phosphate (TCEP) (0.75 - 2.8 ng/m<sup>3</sup>) than previously reported in the literature for similar remote Arctic sites (0.017 – 0.56 ng/m<sup>3</sup>). TCEP was found to be in deposition to Troutman Lake at magnitudes ranging from 290 ng/m<sup>2</sup>/day to 1300 ng/m<sup>2</sup>/day. No PCBs were detected in this study. These findings can help us to understand the fate of anthropogenic contaminants in dynamic Arctic systems with both global and local sources of modern and legacy pollutants.

### 3.06.P-Tu163 Persistent Organic Pollutants (POPs) in *Pygoscelis adeliae* from the Ross Sea (Antarctica): Evaluation of their Temporal Trends in a Climate Change Context

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Antarctica is rapidly undergoing the most pronounced effects of climate change worldwide. Events like ice melting, iceberg calving, and changes in oceanic and atmospheric currents are affecting the lifecycle and health of endemic species, in some cases posing their survival at risk. Among the major acknowledged threats for organism health are Persistent Organic Pollutants (POPs) which are transported to Antarctica by long-range transport mechanisms and which can also be released in local anthropogenic activities. Legacy pollutants that were decreasing in recent years could be remobilised from melting ice and become available for uptake and biomagnification in the trophic webs. Transport patterns of emerging POPs to Antarctica could be changed. Adèle penguin (*Pygoscelis adeliae*) being an intermediate predator and a resident with a circumpolar distribution represents a good sentinel species for monitoring POPs in Antarctica. Continuous monitoring of the presence, distribution and levels of pollutants is fundamental to make predictions about potential consequences of climate change and to evaluate if the implemented protection measures are having the expected effect. That is even more important considering the increasing human impacts connected with scientific research, industrial fishing and tourism activities that could also release contaminants and contribute to contaminant exposure of the penguins. The main aim of this work was to analyse legacy and emerging compounds in penguin eggs from the Ross Sea coast (East Antarctica). Fifty-one unhatched eggs were collected in seven years between 1997 and 2022, during Italian expeditions conducted in the framework of the National Programme of Research in Antarctica (PNRA), and analysed for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and per-/polyfluoroalkyl substances (PFAS). The temporal trends of target compounds will be evaluated and, when feasible, correlations to available climatic and/or biological parameters will be assessed. We hypothesize decreasing trend for legacy pollutants such as PCBs still prevailing on the reduced emissions and increasing trends for emerging POPs such as PFAS due to new environmental inputs or, focusing on specific congeners/isomers, connected with recent human activities.

### 3.06.P-Tu164 Using Faeces as a Noninvasive Biomonitoring of Mercury in Svalbard Reindeer (*Rangifer tarandus platyrhynchus*)

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Anthropogenic activities have increased mercury (Hg) atmospheric emissions, resulting in higher concentrations in the environment. The methylated form of Hg (MeHg) is of high ecological concern, as it bioaccumulates in organisms and biomagnifies in food chains. Even though there are few local sources of Hg in the Arctic, the majority of the Hg detected in the Svalbard biosphere derives from the atmospheric and ocean currents transport.

The study aims to monitor total Hg (THg) and MeHg levels in the faeces of the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) as a non-invasive matrix. *R. tarandus* faeces were collected in Colesdalen, Bjørndalen and Hollendarbukta, Svalbard, during the summer of 2022. The THg content was analysed using a direct mercury analyser (DMA-80), and MeHg levels using MerX-M. The results showed faecal THg levels ranging from 36 – 421 mg/kg dry weight (d.w), depending on location and sampling week. Generally, higher levels of THg were found later in the summer. Faeces samples from Hollendarbukta in the late summer had particularly high levels, ranging from 139 – 421 mg/kg d.w, which is an increase of about 20% compared to the same location at previous sampling weeks. The THg levels in the faeces samples reflect the diet of the reindeer and contribute to the monitoring of Hg levels in the environment and the health of *R. tarandus*. The samples are currently analysed for MeHg levels to evaluate MeHg concentrations and calculate the THg:MeHg ratio. These data provide more information about MeHg distribution in terrestrial ecosystems in Svalbard and its potential for bioaccumulation in *R. tarandus*.

### 3.06.P-Tu165 Mercury Uptake, Distribution and Excretion in Svalbard Reindeer

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Frozen soils of the Arctic have been found to store large amounts of legacy mercury (Hg). Due to climate change and increasing soil temperatures, permafrost is thawing, which could remobilise the accumulated Hg and make it bioavailable for uptake in biota. The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is an important primary consumer, and therefore a key species in the local Arctic terrestrial ecosystem. This study aimed to determine the levels of total Hg (THg, which includes both inorganic and organic Hg) in a range of both tissues and faeces from the Svalbard reindeer, as well as in *Salix polaris*, a major constituent of their diet. These data will contribute to assessing the uptake, distribution and excretion of Hg in an Arctic terrestrial herbivore and form the basis for the potential development of a toxicokinetic model that predicts internal tissue Hg concentrations based on the levels found in faeces.

THg was analysed in *Salix polaris* (n = 15), and fur, faeces, muscle, brain, liver and kidney from twenty reindeer, culled in central Spitsbergen in 2021 (n = 20). All samples were analysed using a direct mercury analyser (Milestone DMA-80 evo). The highest Hg levels were found in kidney (226.55-58.69 µg/kg ww), followed by liver (32.33-120.21 µg/kg ww), faeces (7.33-25.71 µg/kg ww), muscle (0.94-7.64 µg/kg ww), brain (0.04-1.46 µg/kg ww) and fur (0.86-17.65 µg/kg ww). The levels of Hg in *Salix polaris* ranged from 1.95-17.93 µg/kg (ww). Hg levels correlated well between kidney and liver samples and between muscle and brain samples (p<0.001, R<sup>2</sup>=0.77 and 0.95, respectively). While faecal Hg levels represent local dietary intake and short-term exposure, internal tissues such as kidney and liver are known to store Hg, and concentrations in these organs would rather represent chronic exposure. The lack of significant correlation between the faecal Hg levels and the levels in any of the other analysed reindeer tissues is therefore not surprising. The relatively high faecal Hg levels however suggest that while the Svalbard reindeer is exposed to Hg through their diet, they are also efficient in excreting it.

### 3.06.P-Tu166 Legacy and Emerging PFAS in Three Seabird Species in the Sub-Antarctic Using UPLC-HRMS to Evaluate Long-Range Transport and Emerging Chemicals

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Poly- and perfluoroalkyl substances (PFAS) are a large group of chemicals that are characterised by a hydrophilic moiety (e.g. SO<sub>3</sub><sup>-</sup> or COO<sup>-</sup>) and a hydrophobic perfluorocarbon chain that varies in length. PFAS display unique properties due to combined oil and water repellence, extreme stability, and hence are used in a wide range of industrial processes and consumer products. These chemicals can be problematic in the environment due to their potential to undergo long-range transport, persistence and toxicity. However, only some of these substances have been regulated by the Stockholm Convention; therefore, there is a growing need to document evidence of these emerging chemicals of concern in the environment. Here, we employ an ultra-performance liquid chromatograph coupled to a high resolution mass spectrometer (Orbitrap) to analyse a range of both legacy and emerging PFAS including 11 PFCAs (C4-C14), 9 PFASs (C4-C10), 3 FASAs, 3 PFPIAs and 6 fluorotelomer acids, among others. We have analysed liver tissues of black-browed albatrosses, white-chinned petrels and common diving petrels collected from the Falkland islands and South Georgia from the early 2000s to the mid 2010s. The data show varying spatial and temporal trends between different species and compounds; in particular we draw comparisons between trophic levels by employing stable isotope analysis. We have also conducted nontarget analysis to horizon scan for related chemicals that are being released into the environment, and are accumulating at detectable concentrations in biota, but are not on regulatory agendas as of yet. Our research addresses the need to understand exposure levels in wildlife to PFAS compounds in areas close to and remote from sources, and also how PFAS fingerprints vary between different species over time.

### 3.06.P-Tu167 Sources and Levels of Mercury in Terrestrial and Fresh Water Resources Related to Feeding Areas for Reindeer at Svalbard (TERRA)

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Recent studies indicate that permafrost, the permanently frozen topsoil layer, might be the largest reservoir of Hg globally. As a result of Arctic amplification, the circumpolar permafrost soils have started to thaw, and the stored Hg could remobilize into the environment again. Hg is a known toxic pollutant that can cause adverse effects in biological systems, even at low doses. Due to the potential release of Hg from the permafrost, terrestrial species such as *Rangifer tarandus platyrhynchus*, the Svalbard Reindeer, could thus be exposed to Hg through their diet. This study aims to determine the levels of Hg released from permafrost in the Arctic and to compare the sources of Hg uptake for terrestrial species. To assess whether Hg remobilizes from permafrost, gold traps were set at the start of the thawing season and retrieved after summer. Additionally, soil and moss samples were collected to determine the atmospherically deposited Hg. Water and vegetation (*Salix polaris*) samples were also collected. All samples were analyzed using the Direct Hg Analysis (DMA80) for Hg content and ICP-MS for elemental composition. The preliminary results from the gold traps show no significant differences between with the blanks, suggesting that this technique might not have been able to determine the Hg released from the soil during the summer, or that no Hg was remobilized in the studied period. Soil samples from the end of summer show significant location differences, with higher levels of Hg in Colesdalen (178-427 µg/kg), compared to both Foxdalen (53-179 µg/kg) and Bjørndalen (29-64 µg/kg) samples. *Salix polaris* samples show values ranging from 30-172 µg/kg in dry weight. Water levels are still being analyzed. The ongoing hypothesis is that values from both soil and water from the beginning of summer will have lower Hg values than those at the end of summer. This study will



provide an overview of the remobilisation of Hg in terrestrial ecosystems in Svalbard, emphasizing the risk for the Svalbard Reindeer.

### **3.06.P-Tu168 Determination of Organochlorine Pesticides in Biota Using Gas Chromatography Atmospheric Pressure Chemical Ionization (GC-APCI) MS/MS**

**Janitha De Alwis<sup>1</sup>** and Douglas Michael Stevens<sup>2</sup>, (1)Food and Environment, Waters Corporation, United Kingdom, (2)Waters Corporation

Despite the fact that many organochlorine pesticides (OCPs) have been banned or limited in their production and application for decades, their recalcitrance to degradation in the environment and their global transport potential still make them an important class of compounds to monitor today. Furthermore, these compounds bioaccumulate and are associated with a range of negative health effects for many different organisms. So while water and soil samples play an important role in determining the occurrence, fate and transport of OCPs, biota samples from sentinel species more directly connect with the need to determine the incorporation of OCPs into the food web and the potential for human exposure presented by their presence.

Across recent years tandem quadrupole GC/MS/MS with atmospheric pressure ionization has begun to replace electron ionization (EI) on magnetic sectors for challenging environmental analyses such as OCPs. The shift to GC-APCI MS/MS in particular also facilitates the conversion of methods relying on He carrier gas to more sustainable, reliable and less expensive alternative of nitrogen. The atmospheric pressure source adapts to the use of nitrogen carrier gas without loss of sensitivity or chromatographic separation.

This work compares the analysis of whale and seal blubber samples for the presence of OCPs using EI HRMS with He carrier gas to GC-APCI MS/MS using He carrier gas and nitrogen carrier gas. Preliminary data shows agreement between the EI HRMS data and GC-APCI MS/MS within 1ppb and within 10% in the range of 1 to 200ppb.

A calibration curve from 1 - 200ppb was analyzed along with 3 biota samples, 2 interlab study samples, 2 standard reference materials, 2 spiked matrix samples, and 3 blanks. Following the analysis of this batch of samples the system using He and GC-APCI MS/MS the system was converted to nitrogen carrier gas. Across the 49 peaks monitored the agreement between the He 60m column and nitrogen 40m column data averaged +/- 1.1s. Sensitivity between the two data sets showed all analytes within a factor of +/- 2X of the same values as measured using signal to noise. Critical pair separations were the same between the two data sets and all allowed accurate and precise quantitation with good agreement to the EI HRMS data. An alternative method to traditional EI GC HRMS based on GC-APCI MS/MS demonstrated the ability to generate comparable data for the monitoring of OCPs in biota using either He or nitrogen carrier gas.

### **3.06.P-Tu169 Considerations for Designing an Antarctica Monitoring Program for Cyclic Volatile Methylsiloxanes (cVMS)**

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Environmental monitoring in the Arctic and Antarctic remote locations brings several challenges compared to monitoring in metropolitan and major source regions in general. This presentation will highlight important considerations for the design and implementation of a remote monitoring program for cVMS. In particular, the main goal is to be able to obtain accurate cVMS concentrations in air and surface media with a more thorough understanding of impact from local emission sources often found in polar regions and potential bias from collection. In 2021, the Silicone industry partnered with the Norwegian Institute NILU, and launched an Antarctica air monitoring project with a strict quality control protocol and sampling locations that allowed for an assessment of impacts from the local station compared to a sampling location upwind and isolated from the local station. These types of comparison site selections are critical for understanding the impact of these local point sources within the Arctic and Antarctic regions. Impact of local wastewater release or solid waste disposal is equally important.

The types of matrices available also lead to challenges. For example, the concentration in air is much lower than in the Arctic; this requires highly sensitive methods. In addition, soils in Antarctica are often frozen and rocky with low organic carbon (OC); this brings challenges in sample collection and processing. The low OC can lead to difficulty with storage and collections due to high potential for analyte loss.

Use of matrix (soil, vegetation, biota) matched quality control and the use of target analyte spike addition to collected samples and following the exact sample transport, storage, processing, and analytical procedures would be essential to understand loss and contamination during collections, transport, and analysis.

With these considerations a program is being planned in Antarctica for cVMS materials with three main objectives.

- First, to determine the presence of airborne cVMS including fractions both in gas phase and bounded by atmospheric particulates to check if the airborne cVMS concentrations in the remote Antarctica sites are high enough to contribute to exposure in the surface media.
- Second, to determine the cVMS concentrations in soil, vegetation, and aquatic biota in locations free of local source influence.
- Third, to understand the potential of local sources or sample artefacts to impact reported concentrations in this region.

### 3.06.V Climate Change in Arctic and Antarctica and Its Effect on Legacy and Emerging Micropollutants in Abiotic and Biotic Environmental Compartments

#### 3.06.V-01 Brominated and Chlorinated Flame Retardants in the Air and Snowpack of Northern Norway

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Halogenated flame retardants (HFRs) undergo long-range environmental transport with growing evidence of their occurrence in remote oceanic and Polar Regions. In regions of high latitude/altitude snowmelt often represents the dominant hydrological feature, accounting for the majority of surface water flow and a route by which atmospherically-derived pollution can affect freshwater systems. In this study, we examined polybrominated diphenyl ethers (PBDEs) and a selection of current-use HFRs in the winter snowpack in the remote far north of mainland Norway (~69°N). Compounds detected and quantified included BDE-47, -99, -100, -153, -209, allyl-2,4,6-tribromophenylether (ATE),  $\alpha$ - and  $\beta$ -tetrabromoethylcyclohexane (TBECH), pentabromobenzene (PBBz), pentabromotoluene (PBT), *syn*- and *anti*-Decchlorane Plus (DP), and decabromodiphenyl ethane (DBDPE). Concentrations of these chemicals in snow ranged from <1 to >200 pg L<sup>-1</sup> (meltwater), with the highest median concentrations in the order of  $\alpha$ - and  $\beta$ -TBECH>PBBz>PBT>BDE-209>DBDPE>ATE>*anti*-/*syn*-DP>other BDE congeners. Concentrations were considerably lower than those reported from earlier studies conducted in the Canadian High Arctic and on Svalbard (firn/ice core, 2005-2008) indicating rapidly changing sources with time and/or variations in seasonal input. Snowfall scavenging ratios (generated from air samples collected at the same time as the snow sampling) were found in some cases to be high (e.g.  $\sim 2.1 \times 10^7$  for ATE for a fresh snow fall event and  $\sim 10^5$  for other HFRs) indicating the efficiency by which snowfall events can strip these chemicals from the air. Particle-bound fractions ( $\Phi$ ) were significant for the higher MW compounds (e.g.,  $\Phi = \sim 0.5$ -0.8 for DP, BDE-209, DBDPE). Differences in HFR concentrations between the upper and deeper snowpack were observed indicating the propensity of these chemicals to migrate to deeper layers with the onset of melt.

### 3.07.A Coastal Ecosystems are Critical Areas for the Assessment of Pollutant Exposure

#### 3.07.A.T-02 The Role of Plastics in the Bioaccessibility of Trace Elements in New Zealand Near-Shore Coastal Zones

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Plastics represent a diverse range of materials, containing complex chemical mixtures consisting of additives, processing aids, and nonintentionally added substances (NAIS). Chemicals may also be acquired from the environment. The potential risk to the environment that plastics pose, in-part, depends upon these associated chemicals, and how they behave under different conditions, as well as the ecosystem they are present in. A lack of knowledge exists around the levels and behaviour of the trace elements associated with marine plastics in New Zealand, and their bioaccessibility is under-studied worldwide. This study aims to fill this knowledge gap.

*In situ* experiments examined trace elements associated with 5 polymers, of both virgin and artificially aged to represent the type and degree of weathering found in the ocean. Bespoke plastics, with known additives, were attached to marine grade steel frames affixed to pontoons at three distinct locations. Plastics and associated biofilms were sampled over 9 months. Beached debris was collected from across New Zealand. Trace elements were determined for all plastics by ICP-MS. The proportion of each trace element present in the plastic that was bioaccessible to marine invertebrates and vertebrates was determined using two commonly collected items.

Trace elements were detected in the virgin and aged experimental plastics. Cobalt and antimony are used as catalysts in the production of PET, Mn as a degradation accelerant in oxo-LLDPE, and Zn as a colourant in LLDPE and oxo-LLDPE. Antimony was the only element that demonstrated loss over time, with greater loss from aged PET. Sb was not seen to accumulate in the biofilm. A total of 957 plastic items were collected from 21 sites. Trace elements demonstrated a wide range of concentrations. A varying proportion of trace elements were bioaccessible to both vertebrates and invertebrates. Vertebrate gastric simulants leached a greater number of trace elements as well as a higher percentage from the debris.

This study of plastics in the New Zealand marine environments confirms that toxic trace elements are present, and their ability to disassociate from the plastic on ingestion poses a significant threat to New Zealand's unique marine ecosystems. In addition, it highlights that the state of aging is a significant factor influencing the associated trace elements and therefore can affect the behaviour and fate of associated trace metals in the marine environment.

#### 3.07.A.T-03 Searching for Evidences of Seasonal Variation and Plant Effects in the Production of Methylmercury in Saltmarsh Sediments Using Mercury Stable Isotope Techniques

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Mercury (Hg) is a recognized global pollutant and its impact in ecosystems can have dramatic effects. Previous studies have

reported highly Hg contaminated areas in Ria de Aveiro, which makes of extreme importance to understand Hg biogeochemical cycle at a local scale. In saltmarshes, recent studies have suggested that the rhizosphere of halophyte plants play an important role in Hg speciation, more specifically in the Hg methylation.

Throughout the seasons, we evaluated Hg and monomethylmercury (MMHg) natural concentrations and used stable isotope techniques to determine methylation ( $K_M$ ) and demethylation rates ( $K_D$ ). Our aim was to evaluate the impact of plants on the MMHg budget in saltmarshes, understand the impact of seasonal change, and compare two saltmarshes in Ria de Aveiro, a Portuguese contaminated coastal Lagoon.

For this study, one saltmarsh was selected in a contaminated area (Laranjo-LAR) and another used as a reference site (Chegado-CHE). Hg concentrations varied between 0.05 and 6.20  $\mu\text{g g}^{-1}$  in CHE and between 0.07 and 58.53  $\mu\text{g g}^{-1}$  in LAR. These environments also presented significant MMHg concentrations, varied between 2.6 and 334  $\text{ng g}^{-1}$  in CHE and between 2.3 and 260  $\text{ng g}^{-1}$  in LAR. The highest MMHg contents were always recorded in vegetated sediments, which indicates the influence of plant activity in the Hg methylation. Also, in both saltmarshes, the highest MMHg concentrations were recorded during summer, implying that higher temperatures favour MMHg production likely due to higher microbial activity in warmer months. This was also corroborated by the significant increase of  $K_M$  during summer with values up to 0.452  $\text{day}^{-1}$  and 0.312  $\text{day}^{-1}$  respectively in CHE and LAR. Additionally, the elevated  $K_D$  (between 0.48 – 15 in CHE and 1.4 – 32  $\text{day}^{-1}$  in LAR) with significant changes between seasons and highly affected by the presence of plants, indicates that demethylation process happens faster than the methylation process. Higher  $K_D$  values combined with measured half-life of less than 2 days for MMHg (mostly in unvegetated sediments) is surprisingly short. In these particular cases, it indicates that MMHg is not persistent in aquatic systems and a constant supply of MMHg is necessary to maintain a steady level of MMHg in sediments, making the formation process important.

These results showed that the use of stable isotope techniques allowed us to understand the importance of the balance between  $K_M$  and  $K_D$  in the MMHg pool in saltmarsh environments.

### **3.07.A.T-04 Effects of Individual and Binary Mixtures of Aquaculture Pesticides on Nontarget Marine Invertebrates**

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Around 40% percent of global aquatic products derive from aquaculture, showing an increasing trend of production. Salmon aquaculture sites create a particular environment for the aggregation and reproduction of sea lice (marine ectoparasites) which cause severe skin and tissue damage with consequent fish stress, mortality, and economical loss. Chemical solutions involving the usage of drugs and pesticides are commonly used as part of an integrated management plan to maintain fish health and limit loss.

To this end, nearly 75% of the active marine finfish aquaculture sites in Canada use at least one drug or pesticide each year. Chemicals that enter the marine environment are expected to disperse following release.

Despite their wide usage, there are still unknowns and concerns regarding the environmental effects of these compounds, as well as the influence of formulation vs active ingredient (AI) and the impact of mixtures. The formulation ingredients enhance factors such as solubility; therefore the physical-chemical data derived from the AI may not be appropriate when predicting environmental fate and effects. With multiple aquaculture sites using multiple products of differing fates and modes of action, it is important to understand the synergistic, antagonistic, and additive effects of these multiple stressors.

In this study, we performed a series of toxicity tests using the early-life stages of American lobster and green sea urchin with the active ingredient and formulated products of 2 commonly used pesticides, SALMOSAN 50WP (AI: Azamethiphos; recommended dosage: 100 $\mu\text{g/L}$ ) and Paramove 50 (AI: Hydrogen peroxide; 1.2-1.8  $\text{mg/L}$ ).

Immobilization and mortality presented in a concentration-dependent manner within 24-hrs of exposure, with no difference between the formulated product or the active ingredient. The Salmosan:H<sub>2</sub>O<sub>2</sub> mixture trial highlighted possible antagonistic effects between the two compounds.

Significant differences in percent fertilized were observed in the urchin, with a more detrimental effect in the case of the formulation Salmosan, in particular, at the highest concentration. On the other hand, the percentage of normal larvae has not shown differences between the two treatments.

The data generated in this project will provide greater context and understanding of these compounds in coastal environments to define environmental quality standards or monitoring thresholds.

### **3.07.A.T-05 Presence of Microplastics in the Delaware River Estuary in Northeast USA**

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Plastics in the aquatic environment are of increasing concern because of their persistence and effect on the environment, wildlife, and human health. To date, little is known about microplastics in the upper Delaware estuary, USA and their effects on fish and wildlife. The first step in understanding these potential effects is to understand the distribution and concentration of microplastics within the estuary.

This research is aimed to increase the understanding of the distribution of microplastics in the Delaware estuary, USA (a major river in northeast USA) through monitoring and modeling and to increase public awareness of the issues associated with microplastics. Grab and net samples were collected at nine tributary locations and five mainstem locations throughout the basin. Sample analysis was performed using FTIR spectroscopy. Data collected for the samples included total particle count, particle size, color, and composition. A 3-Dimensional hydrodynamics model was applied for the Delaware Estuary using the USEPA supported Environmental Fluid Dynamics Code (EFDC). The 3-D hydrodynamics model simulates hydrodynamic and transport information (e.g., tides, water depth, current velocity, salinity, water temperature) over a range of hydrologic and meteorological conditions.

Microplastics were detected in every sample. Concentration ranged from 8.5 – 250 particles/ft<sup>3</sup> with the highest concentrations occurring in Rancocas Creek. Mainstem Delaware River grab samples generally showed mid-high concentrations of microplastics with similar concentrations being found at both surface and bottom collections. Microplastics were found in a variety of colors in samples including clear, pink, purple, red, orange, yellow, green, black, brown, gray, silver, and white. Apart from this, microplastics were found in sizes ranging from 50 µm to 2000 µm. Grab samples generally had smaller particles with 75% of the particles being less than 500 µm. Net samples had larger particles with 53% of the particles being greater than 500 µm. Samples were composed of a variety of plastics. Polyester, Rayon, and manmade cellulosic fibers were the most common types seen in samples. Microplastic composition was found to be similar between both net and grab samples, however a higher proportion of polyethylene particles were observed in the net samples. Data on microplastic concentrations can be used to target cleanup efforts in high plastic loading watersheds.

### 3.07.B Coastal Ecosystems are Critical Areas for the Assessment of Pollutant Exposure

#### 3.07.B.T-01 Emerging Trace Metal Contamination in Representative Urbanized Estuarine Systems

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Continuously increasing mining, use, and subsequent release of Technology Critical Elements (TCEs) pose growing environmental concerns regarding their dispersion, fate, and ecotoxicological impact in aquatic systems. Platinum Group Elements (PGEs) belong to this group and represent contaminants of emerging environmental concern for which gaps in knowledge remain for their behavior and reactivity in recipient waters, especially in estuarine/coastal systems that are impacted by human activities. Among the variety of specific applications, PGEs are especially used in car catalytic converters (vehicle emission control) as well as anti-cancer drugs (for platinum: Pt), and other industrial processes. While recent studies provided PGE levels in seawater, scarce knowledge still exists regarding PGE contamination in marine organisms, especially for rhodium (Rh) element. Analytical challenges in terms of sensitivity and interference control required for Rh and Pt ultra-trace detection in marine matrices may explain such general lack of information.

In this study, water, macroalgae (various species) and mussels (*Mytilus galloprovincialis*) were sampled in several sites from two estuarine systems and adjacent coastal areas reported to be under anthropogenic pressure, i.e., the Douro and the Ave estuaries (North Portugal). Together with master physicochemical parameters and nutrient levels determined in seawater, Rh and Pt concentrations were quantified through both Adsorptive Cathodic Stripping Voltammetry (AdCSV) and Inductively Coupled PlasmaMass Spectrometry (ICP-MS) in the dissolved phase (< 0.2 µm) and biological samples.

Spatial monitoring of selected PGEs, together with proxies of sewage inputs revealed the presence of Rh and Pt in the dissolved phase as well as in marine biota. The determination of Pt/Rh ratios in the various samples reflected the influence of different PGE sources that include traffic emissions, hospital/domestic effluents, and industrial activities. The higher range of Pt/Rh ratio in the dissolved phase in the Ave Estuary suggested the importance of other Pt-dominated sources that may include specific Pt-based industrial processes. Our study confirmed that urbanized estuaries represent important pathways to transfer PGE from the coastal zone to the open ocean. Further studies are therefore required to evaluate the extent of those emissions and to collect more data on Rh as an emerging coastal contaminant.

#### 3.07.B.T-02 Seals in a Changing Sea: A Multitracer Approach

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We present here an innovative use of stable isotopes ratios of carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulphur ( $\delta^{34}\text{S}$ ) combined with total mercury (THg) and selenium (Se) concentrations in seal body tissues as ecological tracers of harbour and grey seal foraging activities and exposure to Hg. SIBER (Stable Isotope Bayesian Ellipses in R) allows the use of stable isotope ratios, [Se] and [Hg] to build geometric spaces (or “isospaces”). These can be used as proxies of species ecological niches commonly referred to “isotopic niches.” Our objectives are (1) to evaluate potential temporal changes in foraging patterns and exposure of grey seals and harbour seals, (2) to highlight the trophic plasticity of these two seal species in a changing environment.

A multitracers approach ([THg] and [Se];  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values) was applied to harbour seals and grey seals (blood; German and Scottish coasts,  $n = 174$  and  $n = 80$ , respectively) during different time periods (from 1900 to 2017). Additionally, the same tracers were analysed in harbour seals (hair; from 1930 to 2017) from the Natural History Museum of Stockholm ( $n = 10$ ), free-ranging and stranded harbour seals from the North Sea (German and Belgian coasts,  $n = 40$  and  $n = 6$ , respectively). SIBER package in R was used to explore the variation in the isotopic niche ( $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  and  $\delta^{15}\text{N}$  vs.  $\delta^{34}\text{S}$ ) and the ecological niches ( $\delta^{13}\text{C}$  vs. T-Hg, T-Hg vs.  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  vs. T-Hg,  $\delta^{13}\text{C}$  vs. Se, Se vs.  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  vs. Se).

Our multi-tracer approach revealed:

- (1) An increase of the size isotopic and ecological niche size in agreement with a more offshore foraging distribution, and an increase of benthic resources (more flatfish and less sandeels) for Scottish grey seals over time. In contrast, we calculated a smaller isotopic niche size for harbour seals over time likely linked to an increase of phytoplankton biomass, supporting zooplankton for planktivorous fish species such as clupeids.
- (2) Long-term changes having affected the North Sea are reflected in hair collected from harbour seals (1930-2017). We found [THg] and  $\delta^{13}\text{C}$  values increased linearly with time. Higher  $\delta^{13}\text{C}$  values may be related to local higher primary productivity that may explain the possible increase of prey species abundance. Such increases may be related to changes in habitat use, particularly as tracking studies have shown that some foraging now occurs around wind farm installations.

### **3.07.B.T-03 Micro-Estuarines as Bio-Filters: Attenuation of Organic Pollutants and the Effects of Salinity and Seasonality** *Tom Topaz, The Faculty of Marine Science, Ruppin Academic Center, Israel*

Micro-estuaries are small ubiquitous transitional water bodies that mediate surface water to seas and oceans. Unlike the vastly studied large estuaries, micro-estuaries lack the ability to dilute and contain pollution from point and nonpoint sources due to low natural water discharges. Therefore, these diverse ecological systems are susceptible to pollutant loads due to prolonged water residence time and complex geochemical dynamics. Although this elevated anthropogenic stress limits their potential to provide ecological and recreational services, micro-estuaries have some traits similar to those found in constructed wetlands. These traits provide a natural potential to retain and mitigate organic pollutants. A two consecutive years field study conducted at the Alexander micro-estuary tracked the influx and outflux of a large organic pollutant mixture (46 pesticides and 19 pharmaceuticals) during base-flow and flood events. Throughout the research period, 165 kg of active ingredients entered the micro-estuary and 160 kg flowed out to the coastal zone of the Mediterranean Sea, suggesting negligible net attenuation. However, this broad picture conceals seasonal shifts in pollutant mixture loads, and only a handful of pollutants were actually balanced. The majority of compounds were either removed or added to the flow, with no observed correlation to chemical properties. A prominent observation was the load increase along the flow for some pollutants during base-flow conditions. This trend was correlated with salinity elevation and was verified in sorption-desorption lab experiments conducted at different levels of water salinity. These results imply that seawater intrusion to the bottom of the estuary may increase desorption rates of pollutants from the estuary bed, creating an estuarine desorption magnification effect which may increase chronic exposure of the coastal system. The combination of strong anthropogenic stress with increased desorption rates severely limits the estuary's potential to mitigate pollutants, frequently transforming it into a pollution source rather than a sink.

### **3.07.B.T-04 Global and Regional Decrease in Metal Concentrations in Brown Algae**

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Metal pollution is of major concern because of its associated toxic effects and the persistence of these pollutants in the environment. In this study, we evaluated the temporal changes in metal concentrations of coastal brown algae using two complementary approaches: i) A metanalysis of >15,700 metal concentration data across the globe reported from studies for the period 1933-2020; ii) The analysis of >450 samples of *Fucus vesiculosus*, *F. spiralis*, and *F. ceranoides* collected in Galicia (NW Spain, North Atlantic coast) from 1990 to 2021 in the same sampling stations. Both approaches provide different information: large spatial-temporal scale data but with low resolution because of the use of nonstandardized methodologies vs. smaller spatial-temporal scale data but higher resolution given the use of standard methods, respectively. Globally, the study findings revealed significant decreases in the concentrations of Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb and Zn of around 60-84% (ca. 2% annual) in brown algae tissues. The decreases were consistent across the different families considered (Dictyotaceae, Fucaceae, Laminariaceae, Sargassaceae, and Others), and began between 1970 and 1990. Regionally, the concentrations of the metals tended to decrease from 1990 to 2021, although these declines showed oscillations over time. The observed declines could be explained to a large extent by the reduction in metal concentrations in oceans, although global change drivers such as decreasing pH and increasing sea surface temperature and heat content could also explain them partially. In any case, this study shows a reduction in metal concentrations in brown algae over time, which is important in itself given their key role in the trophic transfer of these contaminants. This data can be used as a starting point for future emission scenarios and to inform management decisions.

### **3.07.B.T-05 Sediment Surface Distribution of Organic Pollutants and Ecological Risk Assessment in the South of the Southern California Bight (SCB), Mexico**

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The Southern California Bight (SCB) is a complex ecoregion shared by the US and México on the Pacific Ocean, extending from Point Conception in USA to Cabo Colonett in Mexico. In this work we investigate the presence and distribution of organic pollutants such as PBDEs, PAHs, PCDDs, and PCDFs in surface sediments of this marine environment. We expect that coastal development and economic growth will continue, thereby, increasing the input of these and other pollutants via wastewater into marine sediments threatening the local benthic fauna and food chains.

The sampling design was based on a randomly stratified design using that usually followed in SCB projects. Twenty-six surface sediment samples were collected in 2018 with a Van Veen dredge.

We present data for PAHs, PBDEs and, for the first time, PCDDs and PCDFs in the south end of the SCB. The range of concentrations were from non-detected to a maximum of 9.74 ng/g d.w. and from 3.1 to 312 ng/g for  $\Sigma 14$ PBDEs and total PAHs, respectively. For PCDFs and PCDDs, the concentrations ranged from 0.8 to 9.5 pg/g and from 3.8 to 55 pg/g, respectively. Generally, largest concentrations were found in the samples collected at deeper sites and located to the north and central sampling areas.

All study pollutants were generally found in the sampled places. Except for PBDEs, the other two families of compounds occurred in such low concentrations that they did not appear to represent a large risk to benthic organisms. Nevertheless, since these substances can bioaccumulate and biomagnify, it becomes advisable to investigate on marine food webs if they represent a threat to humans via food consumption.

### 3.07.P Coastal Ecosystems are Critical Areas for the Assessment of Pollutant Exposure

#### 3.07.P-Mo184 Quantifying Temporal Changes in Mercury Speciation and Photochemistry in a Tidal River: Effects of Salinity and Total Suspended Solids

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Daytime volatilization of gaseous elemental mercury ( $\text{Hg}(0)_{\text{aq}}$ ) is an important mechanism for mercury removal from aquatic systems and may potentially limit the formation and bioaccumulation of methylmercury. Changes in incoming solar radiation, dissolved organic matter, salinity, and suspended particles were investigated concurrently with several mercury species ( $\text{Hg}(0)_{\text{aq}}$ , dissolved total mercury (THg), easily reducible mercury (ERM), and mercury associated with total suspended solids ( $\text{THg}_{\text{TSS}}$ )) during daylight hours near the mouth of a hypertidal river, Jijuktu'kwejk (Cornwallis River). An empirical predictive model for  $\text{Hg}(0)_{\text{aq}}$  concentrations was also derived based on experimental lab data for mercury photoreduction in the absence of total suspended solids and assuming negligible photooxidation. There were no consistent temporal changes observed for  $\text{Hg}(0)_{\text{aq}}$  in unfiltered surface water.  $\text{Hg}(0)_{\text{aq}}$  ranged from 0–12 pg L<sup>-1</sup>, THg ranged from 0–492 pg L<sup>-1</sup>, ERM ranged from 13–381 pg L<sup>-1</sup>, and  $\text{THg}_{\text{TSS}}$  ranged from nondetectable–261.32 ng g<sup>-1</sup>. The range of  $\text{Hg}(0)_{\text{aq}}$  predicted by the empirical model was similar to measured THg and ERM concentrations, however it was also shown that ERM was not an ideal predictor of photoreducible  $\text{Hg}(\text{II})_{\text{aq}}$  ( $\text{Hg}(\text{II})_{\text{RED}}$ ). Production of  $\text{Hg}(0)_{\text{aq}}$  appears to largely be suppressed by suspended solids, which limits ultraviolet radiation transmission through surface water. This research demonstrates the importance of complementary field measurements in association with controlled lab experiments, and the need for further research to better understand  $\text{Hg}(\text{II})_{\text{RED}}$ .

#### 3.07.P-Mo185 Examining Export and Bioaccumulation of Methylmercury in a Wetland Impacted by Avian Guano and Water Table Restoration

Nelson J. O'Driscoll, Mia Doncaster, Sara Klapstein and N. Hillier, Acadia University, Canada

Big Meadow Bog (Brier Island, NS) underwent water table restoration which was completed in the summer of 2018. The bog is also host to 4000–6000 herring gulls clustered in a small area in the Northern Bog that feed at nearby mink farming and aquaculture sites. Previous research in our group has shown that bird biomass and waste products are increasing the trace metals and nutrients measured in groundwater. Gull guano analysis results have shown that gull guano is very high in water soluble  $\text{PO}_4^{3-}$  and that total mercury (THg) and methylmercury (MeHg) are low in guano. Research from various studies examining terrestrial freshwater catchment flooding indicated that export of methylmercury (MeHg) increases over a multiyear time span in otherwise undisturbed catchments. An examination of water quality and lower trophic level biota was initiated to set a baseline for methylmercury concentrations in the base of the food web.

Outflow water samples showed a seasonal trend between 2018–2022 with highest concentrations and % MeHg occurring during the summer period for 2019 and remaining lower for 2020–2022 data. The % MeHg increases to >60% in July of 2019 and reduces to <40% in the following years. Similar patterns were observed in DOC (mid summer highs of 40–60 ppm DOC) and total phosphorus (0.5–2 mg/L) with a large portion present as orthophosphate. A survey of invertebrate bioindicators of mercury bioaccumulation in the food web indicated a wide range in concentrations. Highest median MeHg concentrations were observed in water striders, water boatmen, milky backswimmers. An examination of MeHg versus THg results for a subset of samples shows 4% to ~100% of the THg is present in a MeHg form. The top 3 families for MeHg content (Corixidae, Gerridae, Notonectidae) have >90% MeHg likely due to feeding ecology and specific habitat chemistry.

### 3.07.P-Mo186 Geochemical and Ecological Controls on Mercury Speciation and Bioaccumulation in Coastal Invertebrates

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Estuaries are ecologically sensitive areas that are vulnerable to bioaccumulation of methylmercury (MeHg), a neurotoxin that readily bioaccumulates in ecosystems resulting in negative impacts on organism health. The Minas Basin, Bay of Fundy, is an important stopover site for fish spawning and for foraging birds during migration and determining uptake of MeHg by invertebrates at the base of the coastal food web is essential for determining MeHg exposure in these higher trophic level organisms. Fourteen species of invertebrate were collected from five intertidal sites in the Minas Basin during summer 2021 and analysed for concentrations of MeHg and total mercury (THg), and stable isotopes of  $d^{13}C$  and  $d^{15}N$ . The mean concentration of MeHg of all individuals ( $12.78 \pm 11.23$  ng/g dry weight (dw)) was approximately an order of magnitude below the Canadian tissue residue guideline for the protection of wildlife consumers like fish and birds of 157.20 ng/g dw. Trophic position ( $d^{15}N$ ) of all 14 species was strongly positively correlated with bioaccumulation of Hg (THg:  $r_{208} = 0.14$ ,  $p = 0.04$  and MeHg:  $r_{208} = 0.34$ ,  $p < 0.0001$ ). Additionally, stable isotopes of sulfur ( $d^{34}S$ ) (measured in tissues of *Corophium volutator*, *Ilyanassa obsoleta*, and *Littorina littorea*) were positively related to MeHg in invertebrates, and when used in combination with  $d^{15}N$  values improved predictions of Hg concentrations in biota. Trophic Magnification Factors (TMF) for MeHg and THg (1.59 and 1.21, respectively) were similar to others reported in studies of food webs containing higher trophic level organisms. To assess the effects of environmental geochemistry on MeHg bioavailability to invertebrates, organism MeHg concentrations were compared to sediment THg, %LOI (carbon), and  $d^{34}S$ , and porewater concentrations of MeHg, TOC, sulfate, and sulfide. MeHg in porewater was the best predictor of invertebrate MeHg, but sediment  $d^{34}S$ , which can be used as an indicator of sulfate reduction and thus Hg methylation, was also a relatively strong predictor and should be included in studies on Hg bioaccumulation moving forward to improve predictive models. This research provides quantitative data on Hg bioavailability in the Minas Basin which is important for Hg modelling in the region, and can be used to protect both ecosystem and human health.

### 3.07.P-Mo187 Toxicodynamics of Methylmercury in the Saltmarsh Plant *Halimione portulacoides* in Hydroponic Culture

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*Halimione portulacoides* is a saltmarsh plant with a key role on the mercury (Hg) biogeochemical cycle, namely by accumulating high levels of this metal in its roots. Intriguingly, there is still a poor knowledge on the physiological adaptation mechanisms that allow the plant to deal with such high levels of Hg, including of the highly toxic organic form – methylmercury (MeHg). Disclosing the effects of MeHg on the antioxidant defence system could be particularly relevant on the clarification of that topic.

A long-term experiment was carried out under hydroponic conditions, comprising 1, 7, and 14 days of exposure to waterborne MeHg ( $1.84 \mu g L^{-1}$ ). Two different salinities were set (0 and 21 PSU) to explore the interference of this abiotic parameter on MeHg toxicodynamics (biochemical responses related to oxidative stress and photophysiology). Plants were collected in a coastal lagoon (Aveiro Lagoon, Portugal) in summer 2021, namely at a highly contaminated site (Laranjo - LAR) and a reference site (REF), allowing an evaluation of the contamination background interference. Plants were acclimated to the lab for two months before exposure. Leaves and root samples were collected after each exposure time, as well as immediately before exposure (T0) for analysis of antioxidant defences [catalase (CAT), superoxide dismutase (SOD)] and oxidative damage [lipid peroxidation (LPO)], while the photophysiology of the leaves was assessed by pulse-amplitude-modulated (PAM) fluorometry.

Antioxidants displayed variation patterns dependent on the endpoint, but with no clear relation with the plant exposure background. For instance, after 1 day of exposure (at 21 PSU of salinity), CAT activity increased in the leaves of exposed plants both from REF and LAR, while a decrease of SOD was also found after 14 days of exposure (at 0 PSU of salinity) of plants from both sites. Effects of MeHg in the roots seemed to be more prominent when exposure occurred under higher salinity values (21 PSU), as evidenced by an increase of CAT and SOD right after 1 day of exposure, suggesting a fast enhancement of reactive oxygen species. Despite that, no signs of peroxidative damage were detected in any organ, while no photophysiological impairments were recorded in leaves, suggesting that *H. portulacoides* is able to tolerate exposure to MeHg. Changes on enzymatic activities were less evident at 7 and 14 days after exposure, corroborating that conclusion on MeHg tolerance by this saltmarsh plant.

### 3.07.P-Mo188 Application of the Paleolimnological Method to Assess Metal Contamination in Pulp Mill Stabilization Basin Sediments in Coastal Nova Scotia, Canada

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The paleolimnological method is well suited to decoupling geogenic and anthropogenic metal(oids) contributions in Boat Harbour, an effluent stabilization basin located in Pictou County, Nova Scotia. Boat Harbour has been impacted by industrial effluents discharged by a bleached kraft pulp mill (1967 to 2019) and a chlor-alkali plant (1971 to 1992). The former estuary now contains  $>577,000 m^3$  of contaminated sediment, impacted by inorganic and organic contaminants, including metal[loid]s and a

variety of persistent organic pollutants. Previous studies indicated that significant knowledge gaps exist in our understanding of the spatial, stratigraphic, and temporal variation of sediment contamination. Comparison of the geochemistry of effluent influenced contaminated sediment and pre-effluent sediment at Boat Harbour to sediment in nearby reference lakes and estuaries was required to understand the degree to which geogenic and atmospheric sources have contributed to metal accumulation in the effluent influenced sediment. A variety of sampling techniques including lakebed sediment gravity and percussion cores were obtained between 2016-2020 to determine spatiotemporal distribution of As, Cu, Pb, and Zn which consistently exceeded guidelines for aquatic sediments. Results demonstrated that there is no distinct spatial trend in metal concentrations though the effluent was introduced from a point source. Temporally variable concentrations of Cu and Zn in the contaminated sediment are associated with effluent from the pulp mill and chlor-alkali plant but also with atmospheric deposition from regional industrial activity. The source of As is dominantly geogenic; As concentration serves as an effective indicator of pre- and posteffluent influenced sediment. Elevated Pb in the contaminated sediment is the result of atmospheric deposition from combustion of fossil fuels as well as bioaccumulation in the pulp mill effluent feedstock. This study demonstrates that undisturbed, time transgressive samples from both impacted sites and reference sites combined with nondestructive, rapid, small sample analytical techniques such as X-ray fluorescence, provide an accurate assessment of sediment metal contaminant sources and distribution, data required to guide remediation and environmental effects monitoring and compliance.

### **3.07.P-Mo189 *In utero* Maternal Transfer of Heavy Metals in Long-Finned Pilot Whale Mother-Foetus Pairs**

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Long-finned pilot whales from the North Atlantic are highly exposed to heavy metals such as mercury (Hg) and cadmium (Cd). Both Hg and Cd are toxic metals known to cause adverse biological effects, where effects on the nervous system are particularly important, and both liver and kidney can accumulate high levels of both metals leading to damage on these organs. The high metal exposure in pilot whales leads to concern for females transferring the metals to their offspring during pregnancy and lactation, resulting in exposure of the offspring at a vulnerable stage of development, which may lead to subsequent unfavourable effects.

Tissues from pilot whale mother-foetus pairs have been analysed for Hg and Cd to assess the maternal transfer of these metals in pilot whales and their distribution in the body. Muscle samples were analysed for Hg, liver samples for Hg and Cd, and kidney samples for Cd. Muscle and liver samples were also analysed for selenium (Se) because of its involvement in detoxification of Hg (forming HgSe).

The results showed Hg levels in mothers that varied between 1.1 and 2.3 mg/kg ww, whereas the levels in foetuses were between 0.14 and 0.86 mg/kg ww. Hg levels in muscle were found to be between 2 and 12 times as high in mothers than in the foetus (M/F ratios between 1.9 and 11.9), whereas the levels for Hg in liver were between 25 and 325 times higher in the mother than in the foetuses. The Hg concentrations in mothers and foetuses were not correlated in either liver or muscle, but the M/F ratios were negatively correlated with foetus length, reflecting the increased transfer with gestation time. The Hg levels were positively correlated to Se concentrations in liver with higher correlation in mothers than in foetuses.

Cd was not detected in foetus kidney or liver, although the levels in mothers were between 44.7 and 113.2 mg/kg ww, and between 19.4 and 33.4 mg/kg ww in kidney and liver, respectively.

### **3.07.P-Mo190 Stability of the Macrocyclic Gd-DOTA Contrast Agent (DOTAREM) Under Different Estuarine Environmental Conditions**

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Previous studies have reported gadolinium (Gd) enrichment in rivers and estuaries worldwide due to the Gd-based contrast agents (Gd-CA) used in magnetic resonance imaging (MRI). The Gd-CA use has grown exponentially since the end of the 20th century, and since 2005, the number of MRI scans has increased by 50%, with more scans being performed in countries with highly developed healthcare systems. These compounds have been traced in the aquatic environment for over two decades, but their toxicity, fate, and stability under natural conditions are still poorly understood, compromising the risk assessment of these compounds in the environment, but also on human health. Although the high stability in vitro of the macrocyclic Gd-DOTA complex is recognized, little is known about its stability in the aquatic environment.

Gd-DOTA complex was exposed to different estuarine environmental conditions (temperature, pH, salinity and solar radiation) to assess their stability. Gd-DOTA solutions were processed in a seaFAST-pico saline matrix preconcentration and elimination system and Gd concentrations were determined by ICP-MS (Perkin-Elmer NexION 2000C). Results showed that the complex remained stable in fresh, brackish, and saline water environments, even when exposed to extreme temperatures (40 °C) or slightly acidic to basic conditions (6-10), for an exposure period of 96 h. However, a small increase in the free Gd concentration was observed after 18 days when exposed to pH <4, in all tested salinities (0, 18 and 36 PSU), with a degradation increase of up to 29%, after 5 weeks of exposure in freshwater. When exposed to direct solar radiation a low Gd-DOTA degradation (4%) was observed after 24 h at salinity 18 PSU, and remained constant until the end of the exposure period (96 h), while the remaining salinities (0 and 36 PSU) showed negligible values.



These results confirm the expected Gd-DOTA complex's high stability under normal environmental conditions even when exposed to high temperatures. Only a slight degradation was observed when exposed to extremely acidic conditions but no degradation was observed for higher pH values. Regarding exposure to solar radiation, although a slight degradation was observed after 24 h, further studies should be carried out in the future, namely, a combination of other parameters, such as the effect of turbidity and depth on the radiation penetration and consequent effect on the complex degradation.

### **3.07.P-Mo191 Historical Trends of Traditional, Emerging, and Halogenated Polycyclic Aromatic Hydrocarbons in Intertidal Zone from the Yellow Sea Large Marine Ecosystem**

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Historical trends of traditional polycyclic aromatic hydrocarbons (t-PAHs), emerging-PAHs (e-PAHs), and halogenated-PAHs (Hl-PAHs), including chlorinated-PAHs (Cl-PAHs) and brominated-PAHs (Br-PAHs), were reconstructed to investigate contamination status over the last 80 years. Eleven sediment cores were collected from the intertidal zones of the Yellow Sea Large Marine Ecosystem. The concentrations of all target PAHs were highest in Huludao, Yingkou, Tianjin, and Dandong, which are industrial and urban areas, with a great mass inventory at the Bohai Sea. Historical trends of all target PAHs showed similar increasing trends from the 1950s, reflecting the development history and changes in Korea and China, and high molecular weight (HMW) PAHs have increased faster than low molecular weight PAHs. Positive matrix factorization receptor analysis using all target PAHs showed that the main sources of target PAHs were fossil fuel combustion (35% of total concentration), vehicle emission (33%), and biomass combustion (32%). The contribution of fossil fuel combustion continued to increase in all regions, whereas vehicle emission and biomass combustion varied with region and time. The toxicity equivalency quantity (TEQ) value was highest for t-PAHs (more than 99% of total contribution), followed by Cl-PAHs, Br-PAHs, and e-PAHs at all sites and periods. The concentrations of t-PAHs in the Bohai, Yellow, East China, and South China seas have increased significantly since the 1990s, especially, the concentrations increased more rapidly in the intertidal areas than in subtidal areas. The predominance of HMW t-PAHs and about 83% of the total flux were entering the intertidal zone, suggesting that the intertidal zone is important as a buffer zone for marine pollution. Overall, the present study provides novel information on the contamination history and sources of t-PAHs, e-PAHs, and Hl-PAHs at a large scale, along with insights on future pollution prevention and coastal management.

### **3.07.P-Mo192 Passive Microporous Polyethylene Tube Samplers as a Novel Approach to Monitor the Occurrence of Organic Contaminants in L'Albufera Natural Park (Valencia, Spain)**

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Monitoring the presence of pollutants in environmental waters has revealed a large number of emerging contaminants of concern. In surface waters, episodic events such as accidental spills, surface runoff, losses, or deliberate industrial release can be difficult to capture and adequately quantify if water samples are taken at a single point in time that may or may not be representative of the pollutant concentration at the time of sampling. In order to estimate the concentrations of pollutants across a more representative and larger time scale and to be able to accurately assess, passive samplers can provide a complimentary sampling strategy. In the present study, Microporous Polyethylene Tube (MPT) passive samplers were deployed, each containing cylindrical microporous polyethylene tubes with absorbent phases to retain emerging polar pollutants of moderate polarity in 12 sampling points of the L'Albufera Natural Park (Valencia, Spain). The sampling points differ in their hydrological characteristics and/or source of contamination. Passive samplers were concurrently deployed for durations of both 14 and 28 days and grab samples water were collected at different times to generate complementary data and for comparison (deployment day, 14 and 28 days). MPTs were extracted and analysed in the laboratory in order to quantify a wide variety of emerging contaminants, including pharmaceuticals, pesticides, poly- and perfluoroalkyl substances (PFASs) and phosphorus flame retardants (PFRs) using high performance mass spectrometry (HPLC-MS). In addition, contaminant concentrations were compared across the two different deployment periods (i.e., 14 and 28 days). Comparatively higher concentrations of certain compounds, including 2,4-dimethylphenyl formamide, tebuconazole, prochloraz, etofenprox, and PFOS, were observed. Different contaminants profiles (i.e., PPCPs through WWTPs effluents and pesticides from agricultural runoff reaching the wetlands and channels) were observed. MPT derived water concentrations were comparable to those derived from grab sampling techniques ( $p < 0.05$ ,  $R^2 > 95\%$ ). Passive samplers proved effective for water sampling by achieving *in situ* concentrations of a range of analytes of concern in the sampled waters.

### **3.07.P-Mo194 Acute Toxic Effects and Mechanism of Antifouling Paint Particles on Juvenile Rockfish**

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During hull-cleaning process, the antifouling paint particle produced is often directly discharged into coastal seawater and coastal sediment. Chemical component analysis of hull cleaning wastewater has shown that up to 75% of the biocide present is related to

paint particles, which can potentially account for between 6 and 17% of the daily biocide input into a marina. Therefore, antifouling paint particles (APPs) have been spotlighted as a source of metal contamination in coastal sediments, potentially leaching large amounts of copper and zinc into coastal aquatic environments. However, there have been few studies assessing the toxic effects of more complex antifouling system mixtures (such as combinations of heavy metals and biocides) on fishery resources. In the study, we evaluate the toxic effects of antifouling paint particles on juvenile rockfish (*Sebastes schlegelii*) which is one of major fishery resources. Paint particle was sampled from dry dock hull cleaning wastewater and 10-, 100-, and 1000-fold dilutions were exposed for 7 days. Zinc was identified to be the major contaminant with the highest concentration in the wastewater. No lethal toxicity was observed during the acute toxic experiment (7 days) in control and exposure groups. GST (glutathione-S-transferase) and SOD (superoxide dismutase) tended to increase at high concentrations. Hepatic ROS (reactive oxygen species) and brain AChE (acetylcholinesterase) activities were increased after exposure to antifouling paint particles. The gene of metallothionein was highly expressed in fish exposed to the paint particle. Some of immune-related genes including the *G-CSF* (granulocyte colony-stimulating factor), *ISG15* (Interferon-stimulated gene 15), *GST*, and *NF-κB* (nuclear factor Kappa-B) were also increased in fish exposed to antifouling paint particles at 24 h than those of the control group fish. Transcriptome analysis in the brain and liver revealed that the genes associated with metabolism, signaling interaction, and the immune system were significantly varied. Moreover, genes encoding proteins, such as vitellogenin and neuropeptide, were among the top-most differentially expressed genes. These results demonstrate the need for proper management to antifouling paint particles contamination in coastal environment.

### **3.07.P-Mo195 Environmental Fate Modelling of Organic Pollutants From Land-Based and Shipping Emissions (Including Scrubber Water) in the Northern Adriatic Sea Coastal Areas**

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Transitional, coastal, and marine ecosystems are highly complex and linked systems, subjected to a multitude of different anthropogenic stressors, both from land-based sources (e.g., industrial and agricultural activities) and direct discharges (e.g., shipping emissions). Recently, the adoption of the global limits on sulfur content for marine fuels is causing a drastic increase in the use of shipping emission abatement technologies like exhaust gas cleaning system (scrubbers) as an alternative to the use of cleaner but more expensive fuel types, whose acidic exhaust discharge water contain diverse chemical contaminants. For these reasons, the presence of shipping lanes and ports may significantly contribute to the exposure of chemical pollutants in coastal areas, potentially increasing risks posed to aquatic ecosystems and human health.

Fate and transport modelling offers an effective way to estimate the contribution of shipping emissions to the overall exposure to chemicals in transitional, coastal, and marine waters.

In this work an exposure assessment for benzo(a)pyrene and fluoranthene accounting for both land-based sources and shipping emissions was carried out for the Northern Adriatic Sea. The modelling effort, realized within the H2020 “EMERGE” project, focused on the integration of several high-resolution predictive models to help exploring the implications of different abatement emissions techniques in managing environmental risks under current and future scenarios.

In detail, shipping-related emissions of pollutants were simulated with the STEAM (Ship Traffic Emission Assessment Model) model, based on Automatic Identification System data, while land-based emissions were quantified by combining daily river flow measurements with water concentrations from routinely monitoring of each tributary. The newly developed ChemicalDrift model, a chemical transport module part of the open-source Lagrangian framework OpenDrift, was applied for a baseline scenario using 2018 data. Forcing data for the case study area have been obtained from the SHYFEM model (ocean currents, temperature, and salinity) and Copernicus Marine Services (mixed layer depth and winds).

Preliminary results showed that shipping emissions can contribute significantly to the exposure to the selected contaminants, especially during the summer period when water flow from the tributaries reaches its minimum values and the number of cruise ships equipped with open-loop scrubbers increases.

### **3.07.P-Mo196 A Probabilistic Screening Ecological Risk Assessment of Pharmaceuticals and Plant Protection Products in the Venice Lagoon**

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Understanding the occurrence and behaviour of emerging contaminants in coastal areas is paramount to complement the knowledge on “traditional” and regulated pollutants and to achieve a comprehensive evaluation of environmental quality status. Since the risk posed by these substances in the Venice Lagoon has been poorly investigated, a predictive assessment can shape the development of future water monitoring plans.

For this purpose, a screening ecological risk assessment was performed to identify emerging contaminants that either are expected to pose a significant risk or need to be further investigated due to the uncertainty in the available data. Focusing on two classes of emerging contaminants, i.e., plant protection products and active pharmaceutical ingredients, exposure and effects information was deterministically and stochastically combined to screen ecological risks into categories.

Simulation of the environmental distribution and fate of target chemicals in the Venice Lagoon was achieved through the development of a water emission inventory based on sales data in combination with demographic and economic statistics of the lagoon watershed, followed by the application and evaluation of a multimedia level III fugacity model that considers the processes involved in contaminants' natural attenuation. The so-derived Predicted Effect Concentrations (PECs) were then compared with effect thresholds (Predicted No Effect Concentration, PNEC) estimated from existing ecotoxicological data on acute and chronic effects for marine and estuarine organisms, and from additional ecotoxicity tests assessing larval development and reproduction of the copepod *A. tonsa* and larval development of the bivalve *M. galloprovincialis*.

If the ratio between PECs and PNECs supported a preliminary classification of chemicals based on their level of risk (i.e., *low*, *medium*, or *high*), it was with a probabilistic assessment that risk could be better characterized. By considering PEC and PNEC stochastically distributed, a Monte Carlo simulation allowed to incorporate model and data uncertainty into the calculation of a probability of risk for contaminants associated with a "medium risk" according to the deterministic assessment.

This approach could differentiate chemicals that, based on the estimated risk, should be included in future monitoring plans from those contaminants with great data uncertainty for which new ecotoxicity tests on marine and estuarine species are recommended.

### **3.07.P-Mo197 Pollutants in Sediment Cores from Lake of the L'Albufera Natural Park**

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Sediments are excellent archives for studying the long-term variations of pollutants in the environment. For this reason, records derived from the chemical analysis of sediment cores are useful to trace the history of pollutant emissions. This study aimed to assess the vertical variation of organic contaminants and metals in sediment cores collected in two sites of the lake of L'Albufera Natural Park (Valencia) to obtain information regarding historical variation in the composition of sediments. A sediment core sampler (57 mm inner diameter, 1.00 m length; Beeker, Eijelkamp) was used to extract the cores from the lake of L'Albufera. The cores were sampled from boat. The sediment cores were 80–87 cm in length and 5 cm in diameter. The tubes were kept upright in a bucket with ice until they arrived at the laboratory where they were frozen. Once frozen, the tubes were cut into 8 segments of the same thickness (8 slices of 10 cm) using a stainless-steel cutter. Pharmaceuticals, pesticides, poly- and perfluoroalkyl substances (PFASs) were analysed using Orbitrap. The compounds were extracted by different extraction methods and determined both, using wide target screening against a positive list of compounds and non-target screening applying ddMS2 of the 4 more intense ions in each cycle as well as all ions fragmentation. Both positive and negative ionization were used. Also, 24 heavy metals were quantified using ICP-MS, after the appropriate wet digestion extraction procedures. Several organic pollutants, especially pesticides such as Azoxystrobin, Molinate, Tebuconazole, were detected in the sediment in contact with water. Some infiltration of the compounds in the inner layers of sediments were also detected. Regarding heavy metals, their higher concentrations in the more recent sediments reflect increased agricultural and industrial activities. This is supported by the fact that there is an increase in the relative bioavailability of metals as indicated by the values of the total extractable-total ratio when compared to deeper sediments. Superficial sediments provide information on the actual deposited material and the actual status of pollution, but the study of sediment profiles provides information on the historical variation in the composition of sediments settled. These sediments can also be used to examine pollution mechanisms, which are significant for predicting future pollution tendencies and assessing potential environmental risks.

### **3.07.P-Mo198 Assessing Potential Environmental Impacts of Exhaust Gas Cleaning System (EGCS) Discharges from Cruise Line Vessels**

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Emissions from the combustion of high sulfur heavy fuel oil (HFO) have been suggested to increase risks to human health and the environment. Beginning in 2020, the International Maritime Organization International Convention for the Prevention of Pollution from Ships (MARPOL) required vessels to comply with new sulfur air emission standards by: 1) using a very low sulfur fuel oil (VLSFO) or 0.1% sulfur marine gas oil (MGO), or 2) installing an "appropriate exhaust alternative method". To comply with the new standards, most cruise ship operators installed Exhaust Gas Cleaning Systems (EGCS) on their vessels. The EGCS removes 98 percent of sulfur oxides from exhaust air emissions, as well as many pyrogenic and petrogenic PAHs and metals, by spraying ambient seawater into the engine exhaust prior to vessel release. EGCS can be operated in a "closed-loop, i.e., minimal overboard discharge, "open loop", discharging EGCS washwater "buffered" with ambient seawater, or hybrid configuration that can be operated in open- or closed-loop mode. The goal of this study was to evaluate potential environmental risks associated with the operation of open-loop EGCS. A combination of analytical determinations (PAHs and metals), modeling of washwater toxicity and postdischarge environmental concentrations, and validation of model predictions using Whole Effluent Toxicity (WET) bioassay methods were employed. Differences in exhaust gas composition have been attributed to engine load, characteristics of the fuel used, and engine differences (e.g., engine make, size, etc.). Possible differences attributable to fuel type were controlled by limiting the study vessels to a single HFO source with a sulfur content of 2.0-3.5%. Engine energy demand could directly affect fuel consumption and washwater composition. Two engine loads were evaluated in this study, low load ( $\leq 50\%$ , represents "in port operations") and high load ( $\geq 70\%$  represents normal transit operations). A representative set of samples were collected from six ships operated under low and high engine loads. Environmental samples were collected from the seawater

intake, EGCS waters, and overboard discharge for each scenario. Analytical determinations (PAH and metals) were conducted on all samples. Additionally, WET testing was performed for each overboard discharge sample to determine potential toxicity to the organisms in the receiving waters. Results of all determinations will be discussed.

### 3.07.P-Mo199 The Effects of Pyridine on the Activity Behaviour of the Common Shore Crab, *Carcinus maenus*

*Elea Giraud and Alex Ford, University of Portsmouth, United Kingdom*

Pyridine is a by-product of several industrial processes and traditionally extracted from coal tar. In November 2021 a mass stranding of dead, twitching, and moribund crustaceans including edible crabs and lobsters were observed off the northeast coast of England leading to speculation that pyridine released from dredging was the causal agent. This led to campaign groups asking for a cessation of dredging activity in the river Tees until the matter had been resolved. To determine the effects of pyridine on juvenile common shore crabs (*Carcinus maenus*) we conducted a four-day and static renewal exposure experiment with concentrations 1, 10, 100, and 1000 µg/L concentrations. The mortality was recorded and behaviour measured using a Zantiks LT machine after 2 hours, 24 hours, 48 hours, 72 hours, and 96 hours. Concentrations were chosen as below those reported to be lethal but represent some environmentally relevant concentrations recorded in the area over the past decade. Specimens and water were frozen for chemical analysis. Preliminary results have demonstrated that at these concentrations no mortality was recorded following four-day exposure. While the study is currently ongoing, the results will provide a better understanding of the effect of pyridine on marine crustaceans and possibly provide insights on whether pyridine dredged from the coastal sediments could have caused the mass extinction of crabs observed in the Northeast of the UK.

### 3.07.P-Mo201 Plastic Pollution in A Coral Reef Climate Change Refuge

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The Gulf of Aqaba in the northern Red Sea is considered a coral reef refuge from climate change for the coming decades. However, data regarding the magnitude and impacts of plastic pollution on the coral reefs of the Red Sea are greatly lacking. In the present study, we have: (1) quantified and characterized benthic plastic debris, and microplastic (plastic particles < 5 mm), and tested for the prevalence of plastic-associated chemicals among corals, seawater, and sediment; and (2) tested the effects of plastic-associated chemicals on the reproductive products of common coral-reef invertebrates. For this analysis, we used one environmental concentration and one high laboratory concentration of each compound; dibutyl phthalate (DBP), dimethyl phthalate, (DMP), 4-nonylphenol (4-NP), and bisphenol A (BPA).

Our results conclude that compared to coral reefs in other parts of the world, the Red Sea is less polluted by plastic, with  $0.093 \pm 0.091$  item/m<sup>2</sup> of benthic debris, and  $0.55 \pm 0.34$  microplastic/m<sup>3</sup> sampled from the reef surrounding seawater. Other than the negative effect of 4-NP on the settlement of *Rhytisma fulvum* planulae, we found limited effects testing the environmental concentrations. The high 4-NP laboratory concentration (1000 µg/L) treatment had adverse effects on the soft coral *Rhytisma fulvum*, the stony coral *Stylophora pistillata*, the calcifying hydrozoan *Millepora dichotoma*, and the solitary ascidian *Herdmania momus*. BPA at 1000 µg/L had a significant negative effect on the fertilization of the gametes of the solitary ascidian *Herdmania momus*.

The current study substantially extends the current knowledge base regarding the magnitude and impact of plastic pollution in the Gulf of Aqaba. This research provides a scientific base for effective preservation policies and local management strategies aimed at battling plastic pollution and making the reefs of the northern Red Sea a coral refuge protected from both global and local anthropogenic stressors.

### 3.07.P-Mo202 Microplastics in Sediment Samples from the San Francisco Bay Area

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San Francisco Bay Area's sediment is currently monitored for a variety of contaminants; however, data regarding microplastics (MPs) are still scarce. Sediment is expected to be a suitable matrix for monitoring MPs levels trends since it is a likely sink for most of the MPs, regardless of their pathway to the Bay. Even buoyant MPs will eventually sink due to biofouling and adsorption of organic and inorganic materials. Moreover, Bay sediment is also an important matrix for monitoring because sediment may be a source of MPs to the Bay food web. This study analyzed MPs (25 µm to 5 mm) in surface sediment samples (n = 8) and sediment core samples (n = 11). Findings provide a preliminary evaluation of MP levels from different regions of the Bay, as well as an indication of temporal trends. Samples preparation included treatment using advanced Fenton's oxidation, and alkaline digestion, followed by enzymatic digestion and density separation (ZnCl<sub>2</sub>, 1.9 g/mL) in order to remove organic and inorganic matter, respectively. The subsample was subjected to final spectroscopic (µFTIR and RAMAN) confirmation. MPs levels detected along the Bay area ranged from 2.20 MPs/g d.w. to 12.02 MPs/g d.w., with a mean value of 6.38 MPs/g d.w. The most abundant morphology is fibers (66.5%) followed by fragments (22.5%) and films (11%). The sample with the highest MP level was found in the Lower South Bay area, which is expected due to the heavy influence of wastewater treatment plant discharge and urban runoff. The lowest MP level was measured in the North Bay Area. Due to the high heterogeneity in MP levels observed, additional sampling and analysis are necessary to provide clear indications of geographic and temporal trends.

### 3.07.P-Mo203 Chemical Toxicity of Robotic Hull In-Water Cleaning Wastewater on Embryonic Flounder

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In-water cleaning (IWC) involves the removal of biofilms and foulants from the hull of a ship using brushes or water jets. Several factors determine the potential for environmental harm associated with the release of chemical contaminants into the marine environment during IWC, which can create “hotspots” of contamination in coastal areas. To elucidate the potential toxic effects of IWC discharge, we investigated developmental toxicity in flounder embryos, which are sensitive to chemical contamination. Zinc and copper were the dominant metals, while copper pyrithione was the most abundant biocide associated with IWC discharge in two remotely operated IWC. Discharge from IWC carried by both remotely operated vehicles (ROVs) produced developmental malformations, including pericardial edema, spinal curvature, and tail-fin defects. We also analyzed the transcriptomic analysis to verify the potential effects of ROVs cleaning wastewater by Next-generation sequencing. The differentially expressed genes (DEGs) were identified in embryos exposed to cleaning wastewater from ROVs which is analyzed GO enrichment and gene regulatory analysis. In the network, *TTN*, *MYOM1*, *CASP3*, and *CDH2* genes appeared to be key regulators of the toxic effects on muscle development. In embryos exposed to ROV B discharge, *HSPG2*, *VEGFA*, and *TNF* genes related to the nervous system pathway were affected. These results shed light on the potential impacts of muscle and nervous system development in nontarget coastal organisms exposed to contaminants found in IWC discharge.

### 3.07.P-Mo204 Experimental Design and Evaluation of an Artificial Bioreef Colonized with *Ostrea edulis* in the Catalan Coast

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According to FAO, an artificial reef is a submerged structure deliberately placed on the seabed to mimic some functions of a natural reef, such as protecting, regenerating, concentrating, and/or enhancing populations of living marine resources. The objective of this study was to evaluate the colonization of different materials used to construct an artificial biotope. These reefs were built with a concrete base and different complementary materials were added, such as calcium carbonate, *Posidonia* remains, straw, crushed oyster, coconut fibers, rice husks, among other materials. Subsequently, native *Ostrea edulis* larvae and adults were fixed on them in order to create an artificial bio-reef capable of filtering and cleaning the polluted waters of the coast of Tarragona (Catalonia, NE Spain), impacted by urban and industrialized activities as the most important petrochemical pole of the Mediterranean. The colonization of the materials was evaluated by analysing the photosynthetic efficiency (micro-PAM) of the biofilms attached on it and species diversity. Number of individuals attached on and accumulation in fresh tissues of different pollutants such as metals and microplastics evaluated the capacity of filtering and cleaning water by oyster. Results indicate that materials were rapidly colonized by microalgae species up to 8150 organisms/cm<sup>2</sup> (*Licmophora* sp, *Thalassionema* sp, *Cylindrotheca closterium*, *Grammatophora* sp, *Pleurosigma* sp, *Amphora* sp, *Nitzschia longissima*, *Cocconeis* sp, *Navicula* sp, and *Oscillatoria* sp.) and oyster larvae, which these last are not intended for human consumption. The oysters were able to bioconcentrate As, Cd, Hg, Cu, Zn and P, up to 10- to 100-fold with respect to sediments concentration. Oysters also accumulated significant amounts of microplastic particles spanning from 5 to 500 µm size.

### 3.07.P-Mo205 Ecological Effect Assessment of Microalgae by Suspended Sediment: A Study Using Flow Cytometry

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As the demand for marine sand mining increases, it is known that suspended sediment caused by marine sand mining has an adverse effect on marine organisms. However, it is focused only on research for fish or invertebrates with high fisheries value, and studies evaluating the effect on microalgae, the primary producers in marine ecosystems, are unknown. In this study, the effect on cell viability of *Phaeodactylum tricornutum*, *Dunaliella tertiolecta*, and *Isochrysis galbana* are representative diatoms, green algae, and haptophytes, caused by suspended sediment was evaluated using flow cytometry. Growth inhibition, a general endpoint, increased as the suspended sediment concentration in all three species increased. The effective concentration of 50% (EC50), the suspended sediment concentration at which 50% of *P. tricornutum*, *D. tertiolecta*, and *I. galbana* showed growth inhibition, was 12 367, 7666, and 10 719 mg/L, respectively. It was found that *D. tertiolecta*, green algae, was most affected by growth inhibition by suspended sediment. Among the several endpoints of cell viability, chlorophyll-*a* appeared to be more sensitive, followed by esterase activity, cell size, and intracellular complexity. As the concentration of suspended sediment increased, it did not appear to affect cell membrane integrity, which can be presumed to lead to death before being affected by cell size and membrane damage. Exposure to suspended sediment in the marine environment can lead to changes in the overall

structure of the marine ecosystem due to a decrease in food sources to the upper trophic level. These could be used as basic data for estimating the criteria of suspended sediment in the marine ecosystem according to marine sand mining.

**3.07.P-Mo206 Acute Behavioral Changes in Marine Fishes by Exposure to Pile Driving Noise: Indoor Microcosm Study**  
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Underwater noise from anthropogenic activities including pile driving noise during the construction of offshore wind farms has increased in recent decades. Pile driving noise is known to affect the behavior of fish, yet it is limited to some fish species. Here, we investigated changes in behavioral parameters (swimming speed and avoidance distance) of juvenile Japanese sea bass (*Lateolabrax japonicus*), Black sea bream (*Acanthopagrus schlegelii*), and Starry flounder (*Platichthys stellatus*) during the start and end period of pile driving noise exposure. After noise exposure, the swimming speed of sea bass and sea bream showed a negatively significant relationship with sound pressure level ( $r = -0.71, p < 0.01$ ;  $r = -0.32, p < 0.05$ , respectively). Of note, only the sea bass avoided the noise source and avoidance distance was significantly positively correlated with sound pressure level ( $r = 0.70, p < 0.01$ ). Until the end of noise exposure, some fish seemed to habituate to the noise during short-term exposure (1 h). Only the flounder did not show significant changes in both swimming speed and avoidance distance, during the entire experimental period. We verified that hearing sensitivity would be attributed to the presence or absence of a swim bladder, which is closely related to the perception of sound, in accordance with previous studies. Also, the minimum effect threshold of pile driving noise on the behavioral changes of juvenile sea bass and sea bream was confirmed ( $\geq 143.1$  dB re 1  $\mu$ Pa). In further study, other basic data such as physiological parameters (e.g., oxygen consumption rate) for future marine environmental impact assessment will need to be built.

**3.07.P-Mo207 Environmental Risk Assessment – Concept and First Results of the Marine Corophium-Toximeter – Detecting Behaviour of the Marine Amphipod Corophium volutator**

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The detection, environmental risk assessment, and risk mitigation measures against pollution in coastal and marine areas is due to climate change of great importance for the achievement of the UN Sustainable Development Goals 6 and 14 and also in the context of the implementation of EU strategies, such as the Zero Pollution Target for a Toxic-free Environment and the Marine Strategy Directive.

The aim of the study is to develop a sensitive, ecosystem-relevant test system that can be used as a static system in environmental risk assessment as part of the approval of, e.g., veterinary medicinal products in aquaculture and as a continuous monitoring system (early warning system) for coastal and marine waters.

Thus, the commercially available Daphnia-Toximeter (bbe Moldaenke GmbH), which is used for freshwater monitoring with the water flea *Daphnia magna*, is further developed in the present study for estuarine and seawater with the amphipod *Corophium volutator*. The sediment-dwelling *Corophium volutator* is of great ecological importance for the Wadden Sea of the North Sea, occurs also in the Baltic Sea and in estuaries. *Corophium volutator* is used in the standardised acute toxicity test (ISO EN DIN 16712) with the parameter mortality so far. Several studies and review articles have shown that the parameter behaviour in bioassays can be much more sensitive than the parameter mortality. Changes in natural behaviour can have direct or indirect ecological relevance, affecting individual fitness and impairing population dynamics, ecosystem functioning or community structures. A 3D living tube model was developed for the "Corophium-Toximeter", which imitates sediments and their U-shaped living tube. At the current stage of development, artificial seawater as sample water continuously passes through two measuring chambers connected in parallel, each with three crustaceans. The live images recorded with a CCD (couple-charged device) camera are analysed online with an integrated PC with regard to changes in the behaviour of *Corophium*. The method of image analysis enables a series of measurement procedures and plausibility tests to assess *Corophium* behaviour based on various criteria, such as average speed, swimming height, position and fractal dimension (curvature). The "Corophium-Toximeter" is still under development; first results with artificial seawater as negative control and a reference substance are shown.

**3.07.V Coastal Ecosystems are Critical Areas for the Assessment of Pollutant Exposure**

**3.07.V-01 Plastic Pollution Ingestion by Coastal-Nesting Seabirds in the Canadian Arctic**

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Plastic pollution ingestion by seabirds is an increasing issue worldwide, yet different species can vary in ingestion based on various ecological and morphological differences. This provokes the ecological question of which species are better suited for monitoring plastic pollution ingestion across regions and time. In the Canadian Arctic, we examined plastic ingestion in sympatric northern fulmars (*Fulmarus glacialis*), black-legged kittiwakes (*Rissa tridactyla*), thick-billed murres (*Uria lomvia*), and black guillemots (*Cepphus grylle*). These seabird species forage in different parts of the marine environment around the colonies. Here, we present new data and compare to historical work to better inform plastic pollution monitoring in Canada and across the Arctic. In 2021, 51% of fulmars contained plastic, whereas 7% of kittiwakes contained plastic and both murres and guillemots had none. These results are similar to previous years, where surface-feeding species (fulmars and kittiwakes) contained more plastic than

pursuit-diving species (murres and guillemots). Northern fulmars continue to have significantly higher levels of plastic ingestion than other Arctic-breeding species, emphasizing their utility as a monitoring tool for plastic pollution in the Canadian Arctic.

### **3.08.A Current State-of-the Art in Understanding the Occurrence and Implications of Plastics in Terrestrial Environments**

#### **3.08.A.T-01 Validation of Microplastic Accumulation in Agricultural Fields Receiving Sludge from Wastewater Treatment Plants**

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Microplastic is known to accumulate in wastewater treatment sludge in large quantities. The microplastic enters the wastewater treatment plants from urban and household drainage and is concentrated in the sludge during the treatment. Many countries use sludge as a fertilizer, creating a possible pathway for microplastic accumulation in the soil. The extent of this accumulation is, however, still largely unknown.

This study aims to investigate the occurrence of microplastic in the size range of 5000 to 10  $\mu\text{m}$  in fields treated with sewage sludge, to increase the knowledge on microplastic accumulation in such fields and to increase the knowledge on the spatial variation in these fields. In addition, other fields not treated with sludge and samples from nature reserves were analysed and used as baseline.

Samples were collected from a long-term agriculture test fields close to Copenhagen, Denmark. A total of 34 samples were taken from 7 different fields, where 30 samples were collected from three sludge-treated fields. In addition, six samples were taken from other agricultural fields in Denmark which were not treated with sludge, alongside three from Natura 2000 areas.

Approximately 285 g of soil was treated for each sample to extract microplastics. A series of physical and chemical treatments were applied to extract the microplastic from the inorganic and organic matter. Such steps include oxidative treatment, density separation, and enzymatic treatment. The extracted microplastics were analysed using  $\mu$ -FTIR imaging for particles  $<500 \mu\text{m}$ , whereafter the software siMPle was used to analyse the dataset to identify and quantify the microplastics.

Microplastics were observed in all 43 samples, regardless of the origin. The observed concentrations for the sludge-treated samples were, however, higher than other samples, with averages of  $12883 \pm 9153$ ,  $1463 \pm 843$  and  $1431 \pm 516$  counts per kg dry soil for sludge-treated fields, nonsludge-treated fields, and Natura 2000 samples, respectively. A high standard deviation was seen for all samples, indicating that microplastic is not easily homogenized when entering the terrestrial environment. This also amplifies the importance of sampling strategy, especially if the sampling is done for monitoring purposes.

#### **3.08.A.T-02 Investigating the Co-Occurrence of Macroplastics, Microplastics, and Plasticisers in UK Soils**

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The risk posed by plastic waste to terrestrial organisms depends in part on the plastic additives which are released from the polymer matrix during the lifetime of a plastic. Plasticisers are a widely used class of plastic additive. Concerns have been raised in recent years over the endocrine-disrupting potential of some legacy plasticisers (phthalates) which has led to an increase in the use of nonphthalate alternatives (emerging plasticisers). However, there remains a lack of empirical data concerning the relationship between plasticisers and macro- and microplastics in soils.

We conducted a field study with the aim of assessing the co-occurrence and potential sources of macroplastics, microplastics, and legacy and emerging plasticisers in UK soils. Surface plastics (macroplastics) and microplastics (25-567  $\mu\text{m}$ ) were characterised and quantified using ATR-FTIR and  $\mu$ -FTIR spectroscopies. GC-MS was used to quantify 8 phthalate and 3 emerging (adipate, citrate, trimellitate) plasticisers.

Sites adjacent to landfill and urban roadsides exhibited the highest concentrations of contaminants. Polyethylene (PE) and polypropylene (PP) were the most commonly detected surface plastic polymers (both 47% detection frequency, DF). PE was also the most commonly detected polymer in the microplastic fraction (21% DF), although PP had the greatest mean concentration of all microplastics ( $7.0 \pm 17.2$  particles  $\text{g}^{-1}$  dw). Total mean plasticiser concentrations in urban roadside, landfill, and urban parkland sites were 29.9, 2.5, and 1.4 times greater than in woodland sites. Diethylhexyl phthalate was the most abundant plasticiser (mean concentration  $390.0 \text{ ng g}^{-1}$  ww, 63.2% DF). The most commonly detected plasticisers were di-n-butyl phthalate (mean concentration  $15.4 \text{ ng g}^{-1}$  ww, 94.7% DF) and the emerging plasticiser trioctyl trimellitate ( $13.5 \text{ ng g}^{-1}$  ww, 89.5% DF).

Based on the data from this field study, phthalates remain the dominant plasticisers in soils, although emerging plasticisers were detected across all land uses and in comparable concentrations to phthalates. These findings raise questions regarding the accumulation of emerging plasticisers in soils as society shifts away from phthalates. This study represents the first investigation of macroplastics, microplastics, and legacy and emerging plasticisers co-occurrence in field soils.

#### **3.08.A.T-03 Microplastic Interaction with Soil Water - Visualization and Quantification with Dual Neutron and X-Ray Imaging**

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Soil is considered the largest sink of microplastic in terrestrial ecosystemsthough little is known on implications of microplastic for soil. Since microplastic is initially hydrophobic, crucial for its fate in soils is the interaction between microplastic and water. If microplastic is translocated by water flow and, vice versa, microplastic impacts water flow - to what extent? This is also important for evaluating the degradation of microplastic, as water is needed for colonization by microorganisms. To approach this question, simultaneous imaging of microplastic and water in soils was utilized.

Dual neutron and X-ray imaging at the beamlines ICON and NEUTRA (Paul-Scherrer-Institute) during wetting-drying cycles was applied to trace microplastic-water interactions in aluminum containers filled with sand and microplastic. Simultaneous neutron and X-ray tomography was utilized to capture the initial microplastic configuration in samples. Subsequently, neutron radiographies of deuterated water flow through the sample were recorded. After drying, repeated tomography gave insight into microplastic translocation.

Imaging results showed that regions of major microplastic content are water repellent. Water flow bypasses and microplastic is mainly retained. Resultant air entrapments lead to reduced water contents. In regions of minor microplastic content water can infiltrate. Here, the air-water interface collects isolated microplastic and shifts their distribution towards an enhanced aggregation. Extrapolation of these results to natural soil systems suggests that less water at regions of high microplastic content might limit their degradation due to reductions in hydrolysis, coating, and colonization by microorganisms.

### **3.08.A.T-04 Effects of Particle Size and Surface Charge Density on the Transport of Nanoplastic Particles Through Porous Media Under Unsaturated Conditions**

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The use of plastic in the agricultural practices can lead to the formation of micro- and nanoplastics (MNPs) by fragmentation and degradation processes. The mobility and transport of these particles towards deeper soil horizons may be influenced by their inherent physicochemical properties and the occurrence of unsaturated conditions. Modelling approaches investigating transport of MNPs through porous media under such conditions are relatively scarce. Therefore, in this study we explored the effects of particle size and surface charge density on the transport of polystyrene nanoplastic particles (PSNPs) through sand with the aim of quantifying such effects through numerical modelling techniques. PSNP infiltration experiments under unsaturated conditions were run in triplicate using an *ad hoc* designed system with glass columns. After a hydrochemical equilibration phase, fluorescent COOH-PSNPs (40 mg L<sup>-1</sup>) were injected over the sand top surface at a flow rate of 0.5 mL min<sup>-1</sup>. The effluent was collected every 30 min and PSNP concentrations were measured with a UV-VIS spectrophotometer. The hydraulic transport parameters of the sand were determined with a tracer test performed after PSNP infiltration. Additionally, PSNP transport through the porous media was simulated using ColloidFIT and COMSOL Multiphysics software. The DLVO theory was applied to calculate the total interaction energy between PSNPs and sand surface assisting in data interpretation. Results show that smaller particles reach higher plateau concentrations than larger particles indicating an influence of particle size on the mobility of PSNPs. Mass recovery percentages from each PSNPs confirm that larger particles tend to be more retained in the sand column. Preliminary modelling results show sorption-desorption rates that point at higher retention capacity for larger particles. According to calculated energy interaction forces, electrostatic repulsive forces between sand and PSNPs seem to be predominant under the experimental conditions, and particle retention should be more likely due to physical constraints in the sand pore spaces rather than chemical sorption processes. Regarding the surface charge density, a variation by a factor of 3 of this parameter does not influence the fate of PSNPs. Further experimental and numerical modelling is on-going and will help to gain further insights into MNP transport in porous media and the relevance of different processes on groundwater contamination

### **3.08.A.T-05 Microplastics in Soil Systems, FromSource to Path to Protection Goals**

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Microplastics can be found everywhere in the world, and in any environmental compartment. During recent years it was discovered that most microplastics may end up in the soil compartment. While the amount of research on microplastics in the soil compartment has increased almost exponentially in the last 5 years, the number of scientific publications are lagging behind these on microplastics in the water compartment. There are indications that microplastics pose a risk to human health and soil life. Therefore, to inform policymakers and risk assessors, RIVM has summarized current knowledge and identified knowledge gaps of microplastics in the soil system. This poster presents the results of this inventory using a conceptual model which addresses topics considered most important for risk assessment. While the model includes several topics, the information presented here focusses on the topics sources, release and emission, fate and transport, and exposure.

### **3.08.B Current State-of-the Art in Understanding the Occurrence and Implications of Plastics in Terrestrial Environments**

#### **3.08.B.T-01 Biodegradable Mulch Films in Agricultural Soils: Analytical Advancements and Biodegradation Dynamics Across Incubation Scales**



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The market share of biodegradable mulch films is increasing. These films offer an alternative to conventional polyethylene mulch films and, thereby, help to overcome soil plastic pollution. The current European norm for biodegradable mulch film stipulates that at least 90% of the film polymer carbon is converted into CO<sub>2</sub> (either in absolute terms or relative to a biodegradable reference material) over a period of two years in laboratory soil incubations. Research is thus needed to understand the factors that govern biodegradation processes and to ensure that biodegradable mulch films indeed biodegrade adequately also in the field in situ. To address these needs, comparative field and laboratory incubations are needed for different soils. This study aimed to a) advance an existing methodology using Soxhlet extraction and proton NMR analysis to quantify residual polymer(s) in soils and subsequently apply this methodology to b) systematically compare biodegradation dynamics of poly(butylene adipate-co-terephthalate) (PBAT) and polylactid acid (PLA) based biodegradable mulch films in laboratory, greenhouse mesocosm and field incubations. Biodegradable mulch film samples as well as positive and negative biodegradation reference materials were incubated at the above-mentioned scales and in three different soils and sampled every ~4 months for a total period of 2 years to quantify remaining polymers. We find that biodegradation rates of PBAT and PLA were strongly soil dependent and that significant differences existed in biodegradation rates and extents between laboratory, greenhouse mesocosm and field incubations. The field incubations showed lowest biodegradation rates not only for PBAT and PLA but also for the positive biodegradation reference polymer. The results support that soil pH, temperature and moisture determine biodegradation because these factors control microbial community composition, microbial and enzymatic activities as well as ester bond hydrolysis of PBAT and PLA. Our study further shows that soil pH could be used as an indicator for biodegradation potential of soils. Furthermore, the results highlight the importance of including positive biodegradation reference materials in incubation experiments, particularly at the field scale. More comparative studies across scales are needed to allow for the prediction of field in situ soil biodegradation rates of polyesters based on their laboratory biodegradation in the same soils.

### **3.08.B.T-02 Effects of Starch-PBAT Blend Microplastics on Soil Organisms, Microbial Functioning, and Soil Physicochemical Properties in a Mesocosm Study**

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A large proportion of the estimated 722 kt of agricultural plastics placed on the European market annually ends up in the soil, breaking down into microplastic (MP). To assess the environmental risk of MPs, single species tests are performed, but results are difficult to translate into effects on complex ecosystems. In this mesocosm study, a small ecosystem was recreated to obtain insight into more complex effects of MPs in agricultural soils, like on soil microbiome, physicochemical properties, plants, and invertebrates. Soil columns were filled with Lufa 2.2 soil moistened at 50% water holding capacity, spiked at MP concentrations of 0.025%, 0.05%, 0.2%, and 0.8% (w/w dry soil). Controls were included. The soil cores were preincubated to allow microbiome development for two weeks prior to the start of the experiment. Cress (*Lepidium sativum*) and lettuce (*Lactuca sativa*) were grown in the mesocosms. At t = 0, 4, 8, and 11 weeks lettuce was sampled for analysis of shoot length, fresh and dry weight, number of leaves, specific leaf area, pigmentation, oxidative stress, and antioxidant enzymes. Three species of Collembola, *Sinella curviseta*, *Heteromurus nitidus*, and *Protaphorura fimata*, were introduced into the mesocosm, to assess effects on community composition. One subadult and one adult of two earthworm species, *Lumbricus rubellus* and *Aporrectodea caliginosa*, were introduced, and checked for survival and reproduction at the end of the experiment. Earthworm survival was not affected by MP exposure, but the total number of juveniles at 0.2% and 0.8% was significantly lower than at 0.025% and 0.05% MPs in the soil. Soil pH (0.01 M CaCl<sub>2</sub>) was significantly (p<0.001) affected at all MP concentrations after 15 weeks (2 weeks incubation and 13 weeks experimental length). Although the exact mechanism behind this pH change is yet to be determined, it may have implications for, e.g., soil invertebrate performance and abundance or nutrient availability. Yet to be determined is whether soil pH is a possible consequence or explanation of the observed effects on microbial activity. Potential ammonification (PAMO), potential nitrification (PAO), basal respiration (BR) and substrate induced respiration (SIR) were all affected by the presence of starch-PBAT blend MPs. This mesocosm study shows the added value of testing more complex systems than single species tests, as many effects were observed at environmentally relevant concentrations of agricultural microplastics in soil.

### **3.08.B.T-03 Pure Micro- and Macro-LDPE and PP Plastic Negatively Affect Crop Biomass and Nutrient Cycling, and Reduce Soil Moisture and Microbial Biomass with Increasing Concentration**

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The intense use of agricultural plastic in the form of mulch films over the last 50 years has led to an accumulation of plastic in soil over time, creating a legacy of plastic in agricultural fields. Plastic often contains additives to provide it with the desired properties, e.g., durability, flexibility; however it is still largely unknown how these compounds affect soil properties, potentially influencing or masking effects of the plastic itself. Therefore, in this study the aim was to investigate the effects of pure plastics in

varying sizes and concentrations, to improve our understanding of plastic-only interactions within soil-plant mesocosms. We measured the effects of plastics on key soil (water content, microbial diversity and biomass, soil nutrient content, enzyme activity) and plant properties (growth, chlorophyll content, nutrient content). Maize (*Zea mays* L.) was grown with typical nutrient inputs over eight weeks with addition of pure micro and macro low-density polyethylene (LDPE) and polypropylene (PP) with increasing concentration (equivalent of 1, 10, 25, and 50 years agricultural mulch film use). We found the effect of both macro- and microplastic on soil and plant health is negligible in the short-term (i.e., 1 to <10 years of plastic application). However, for  $\geq 10$  years plastic application there was a clear negative effect on plant growth for both macro and micro sized LDPE and PP. Here, we provide vital insight into the effect of both macro- and microplastics on soil and plant properties. Future work is needed to investigate the interaction between macro- and microplastic on soil and plant health, as well as how the findings of this mesocosm study translate to larger-scale field conditions.

#### **3.08.B.T-04 Plastic Mulch and Pesticides Residues Effecting the Lettuce Growth: The Soil Microbiome Perspective**

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In arid and semiarid regions, the use of plastic mulch and pesticides in conventional agriculture is nearly ubiquitous. The most common plastic used for mulching is Low Density Polyethylene (LDPE). LDPE mulch needs to be removed after harvest and LDPE debris accumulates in the environment. Some plastic producers have tried to improve the degradation processes of plastic to avoid plastic mulch removal and plastic debris accumulation by adding pro-oxidant additives to LDPE, so called Pro-oxidant Additive Containing (PAC) plastics or by using biodegradable plastic polymers. However, PAC plastics leave residues when ploughed into the soil and some biodegradable plastic do not degrade as fast as expected in the field conditions. Similarly pesticides are known also to accumulate in the soil.

Studies investigating the effect of these contaminants on the soil fertility most often consider pristine plastic or single pesticides. We investigated the effect of one year incubation of plastic debris and pesticides in soil on the growth of lettuces in south east Spain. More precisely, we tested three plastic mulch residues and 3 commercial pesticides, including a treatment with the cocktail of the 3 pesticides compared to controls for a total of 20 treatments. The plastic mulches were first exposed 3 months to the summer weather in the field, cleaned, cut, grounded into macro- and microplastics debris and added in 1kg soil mesocosm at a 0.2% content w/w. Pesticides were applied 3 times, every 6 months, following the prescribed application doses. The 1kg soil mesocosms were left 1 year, protected from the wind and were irrigated to recreate farming conditions. After one year lettuce seedlings, *Lactuca sativa*, were planted in the incubated soil. The plant growth was assessed during the growing period (number of leaves, chlorophyll content, stomatal conduction) and at the harvest (fresh and dry biomass, leaf area, stem diameter). The soil chemical properties were analysed again. Soil samples were collected for analysis of the bacterial and fungal through 16S and ITS sequencing. Results indicate that lettuces growing in soil incubated with the tested biodegradable plastic produced a significantly lower biomass compared to the control or the soil incubated with LDPE plastic. The microbiome data is being analysed and will be presented during the conference to explore why biodegradable plastic led to less biomass production.

#### **3.08.B.T-05 Disentangling Microplastics Effects on Oxygen Diffusion, Microbial Activity, and Greenhouse Gas Emissions**

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Understanding the responses of soil ecosystems to the presence of microplastics (MPs) becomes important as multiple stressors can act together to impact this environmental compartment. MPs impact soil structure, nutrient cycling, and greenhouse gas (GHG) emissions. However, the mechanisms underpinning the direction and magnitude of MPs effects on these soil properties are uncertain, mainly due to the lack of knowledge of how the presence of MPs drives changes in soil structure and the subsequent linkages between soil structure and microbial activity. Here, we hypothesized that the presence of MPs affects soil structure, affecting pore connectivity, leading to higher or lower O<sub>2</sub> availability and consequently higher or lower soil respiration. Furthermore, we anticipated that the magnitude or direction of the effects would be dependent on soil texture and the shape of MPs. We spiked PET MPs fibers (500  $\mu\text{m}$  length) and fragments (125 – 250  $\mu\text{m}$ ) into custom built rhizotrons (5 x 5 x 1 cm) filled with either clay or sandy loam soils (contrasting textures) with a MPs treatment of 1 w/w%. First, we determined the differences in pore connectivity in air dried soil using the rate of O<sub>2</sub> diffusion as a proxy. Next, we determined the O<sub>2</sub> concentration in the soil in optimal conditions for respiration (50% water holding capacity and addition of 20 mg g soil<sup>-1</sup> of glucose). O<sub>2</sub> diffusivity and O<sub>2</sub> concentration were determined by mapping O<sub>2</sub> concentrations using optode imaging, over the course of 48 hours. Fluxes of CO<sub>2</sub> were measured under the same conditions by placing replicate set-ups in a Tedlar bag and collecting gas samples for estimates of CO<sub>2</sub> concentration using gas chromatography. O<sub>2</sub> diffusion was faster in the sandy loam compared to the clay soil. The addition of MPs reduced O<sub>2</sub> diffusion rate in the sandy soil but increased it in the clay soil. In optimal conditions for soil respiration, the treatments showed the same trend for O<sub>2</sub> concentrations, suggesting that O<sub>2</sub> concentration in the soil matrix is likely to be regulated by pore connectivity opposed to respiration. Collectively, we show the impacts of MPs addition to soil and their impacts on the linkages between soil structure, microbial activity and GHG emissions. This study can serve as a baseline for understanding the important impacts of MPs have on soil functioning, which is relevant as plastics are increasingly used directly in agriculture and consequently have direct releases to terrestrial ecosystems.

#### **3.08.P Current State-of-the Art in Understanding the Occurrence and Implications of Plastics in Terrestrial Environments**

### 3.08.P-Tu170 Assessing the Impact of Microplastic Extraction Methods on Biodegradable Polymers

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Agricultural soil is a sink for microplastics (MPs), with both indirect and direct sources. Indirect sources include the use of sewage sludge as fertiliser, plastic mulching films, atmospheric deposition, irrigation with wastewater, etc. It is difficult to quantify the MPs addition from these sources, but the use of sewage sludge as fertiliser is estimated to add 63-430kt/year of MPs into European farmland.

In comparison, the direct addition of MPs to soil can be quantified. In the EU, direct sources (e.g., the application of controlled release fertilisers, and plant protection products) account for approx. 23,500 tonnes/year of MPs, of which plant protection products account for approx. 1,000 tonnes/year (ECHA, 2019). This is preventable and under new restrictions proposed by the European Chemicals Agency, biodegradable polymers are suggested as future alternatives (ECHA, 2019). It is likely that biodegradable polymers will be used in soil applications and therefore essential that robust extraction and analytical methodologies are developed to monitor their environmental fate. However, such methods for the analysis of MPs in soil have not generally been designed for biodegradable polymers. For environmental soil samples, a combination of density separation with  $\text{ZnCl}_2$  and enzymatic-oxidative digestion is a promising technique that has been shown to have a high removal efficiency for soil organic material (e.g., Möller et al. 2020).

In this work, enzymatic digestion has been tested on representative polymers polylactic acid (PLA) and poly(r)-3-hydroxybutyrate (PHB). Polymer films were made from powder, then their stability after exposure to the enzymatic-oxidative digestion method was determined. Impacts of each treatment were assessed visually via microscopy and quantitatively by weight and thickness measurements. Surface degradation was measured with attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), and polymer molecular weight was measured by gel permeation chromatography (GPC).

Results show variability in the impact on the polymer depending on the treatment used and the application sequence. A combination of both visual assessment and chemical characterisation was needed to determine impacts on polymers. This work highlights the importance of quantifying the impacts of microplastic extraction methods on specific polymers of interest to improve future method development.

### 3.08.P-Tu171 Suitability of Elutriation for the Extraction of Microplastics from Natural Soils

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We evaluated the suitability of elutriation for improving the extraction of microplastics as a function of soil textures. Microplastic pollution in marine environments has been extensively studied in modern literature; however, information about the prevalence of microplastics in freshwater and terrestrial systems is lacking. The lack of published literature regarding the extent of microplastic contamination of terrestrial ecosystems is in part due to the lack of any standardized extraction methodology for microplastics from high-clay and organic-rich soils. In this study, we obtained five samples with textures varying from, 0.1-8.6% organic matter, 16.7-96.3% sand, 2.5-45% silt, and 1.3-50.0% clay and compared the extraction efficiency of an extraction process that included elutriation with a typical direct digestion process. All soils were subjected to microplastic extraction with and without elutriation (direct digestion). Samples were digested in 7.5%  $\text{NaOCl}$ , then flotation in 5.1M  $\text{ZnCl}_2$  solution. The number of particles was quantified through staining and fluorescence microscopy. The amount extracted varied from 3300-188 900 particles/kg in elutriated soils and from 3600-153 700 in direct digested soils. Linear regression was used to determine the relationship between relative number of particles extracted (with elutriation vs. without elutriation) and combinations of soil parameters. Correlations were observed between relative extraction quantity and clay content ( $R^2 = 0.64$ ), (clay+OM)/(silt+sand) ( $R^2 = 0.69$ ), (clay+OM)/silt/sand ( $R^2 = 0.81$ ), (clay+OM)<sup>1/2</sup>/silt/sand ( $R^2 = 0.88$ ), suggesting that elutriation may be effective as an extraction step for high-sand and silt soils, and should be avoided for high-clay and organic matter soils. Currently, we are validating the plastic composition of the particles using Attenuated Total Reflectance – Fourier Transformed Infrared spectroscopy and quantifying the amount of remaining metals and salts using Inductive Coupled Plasma Mass Spectroscopy and Emission Dispersive X-ray spectroscopy. The information gathered in this study should help other groups in determining the extent of microplastic contamination in Earth's ecosystems by providing viable extraction methodologies for a range of soil types. A rigorous understanding of microplastic prevalence in the environment will aid in determining the impacts associated with microplastic contamination.

### 3.08.P-Tu172 Shifting to Terrestrial Ecosystems: Characterization of Microplastics in Organic Waste Amendments

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Microplastics (MPs) have been identified as a pollutant of emerging concern and are ubiquitously detected in the natural

environment. One of the major pathways of MPs entering terrestrial ecosystems is through the fertilization of soils with organic wastes. Using organic wastes as soil amendments has some benefits for soil structure, water retention, soil nutrients, organic matter concentration, and soil microbial communities. The aim of the present study is to identify MPs size, morphology, and composition in differently treated organic wastes used in agricultural soils and/or for soil restoration. The organic amendments analyzed ( $n = 7$ ) are sewage sludge, horse manure, two composts, and two digestates from agri-food industries and selectively separated municipal organic waste, and a biostabilized product from nonselectively separated municipal organic waste. The samples in the laboratory were subjected to pretreatment that consisted of advanced Fenton oxidation, alkaline, and enzymatic digestion, and density separation followed by spectroscopic confirmation. Preliminary data suggested that MPs levels in organic waste amendment samples ranged from 472,324 MPs/kg to 7,142 MPs/kg depending on the origin and the treatment of the organic waste. The most abundant morphology is: fragments, followed by fibers and films. The polymer composition of fragments larger than 0.5 mm was mainly made from PET, PE, and PS; fibers of PES, acrylic, PE, and PA; and films of PE, PP, PVC, and PES. This preliminary investigation is suggesting that MPs are highly presented in organic waste amendments and are a significant source of MPs to agrosystems and restored soils. When organic amendment containing MPs is applied to the soil it can be ingested by the terrestrial biota, entering the food chain, and posing a risk to human health. Samples after anaerobic digestion treatment contain the highest MPs concentrations compared to horse manure which has the lowest. Additional study is needed to fully comprehend the threats that microplastics in organic amendments bring to human and environmental health.

### **3.08.P-Tu173 Assessing the Plastic Contamination in Agricultural Soils: A Protocol from Nano to Macro Implemented in 220 Fields Across Europe**

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Plastic use in agriculture has tremendously increased in the past decades resulting in plastic contamination in soils. Several studies intended to quantify the level on the plastic contamination in soil, facing the limitation in size, plastic density, and polymer types. For the project MINAGRIS, a monitoring plan is implemented in 11 Case Study Sites (CSS) across Europe, combining four different protocols to quantify nanoplastics, microplastics, mesoplastics, and macroplastics contamination in soil. The sampling range to 0.1 m<sup>3</sup> of soil searched for macroplastics, 500g soil for mesoplastics, 5g soil for microplastics and nanoplastics. Macroplastics and mesoplastic will be quantified by weight, area, and single-point ATR-FTIR. Microplastics will be quantified with Vibrational spectroscopy imaging with MCT FTIR (LUMOS II) and FPA FTIR (Cary 620 FTIR). The identification method for nanoplastic is still under development.

We will present how combining several sampling strategies and analysis protocol we can assess a wide size distribution of plastic debris in soil.

### **3.08.P-Tu174 Detection and Characterization Methods for Micro(Nano)plastics in Soil**

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In the last decades, the use of plastics across all sectors of the economy has generated a global contamination of plastic waste. In the case of environmental soil residuals of mulching in the form of micro(nano)plastic (MNPs) are a major contributor to soil pollution.

To evaluate this contamination, an extraction method of MNPs from soil based on reducing the organic matter content and separating plastic particles from inorganic parts by centrifugation was developed. Specifically, this study was focused on polymeric particles with different densities: polyethylene (PE) and polyvinyl chloride (PVC). As a first step, stability in the digestion reagents, flotation medium and centrifugation time were assessed and optimized. The effect of each factor on nanosized particles was observed by Particles Extinction and Scattering (SPES).

The optimized extraction method was then applied on soil spiked with microplastics, namely commercially available round shaped fluorescent PE, in-house synthesized fluorescent PVC, PE and PVC uncolored fragments. For the first two plastic types, recovery was determined by fluorescent microscopy, while for the latter the Nile Red (NR) staining method was applied to colour particles with the fluorescent dye and being able to distinguish them from inorganic materials under a blue light. The chemical identification was performed by Raman spectroscopy ( $\lambda = 785$  nm).

The results of this study suggest that NR staining does not interfere with Raman analysis when the sample was spiked with pristine particles. However, some limitations may occur with environmental sample since natural aging processes in the environment may oxidize the particles. Regardless, the extraction method developed in this study proves to be a good protocol for the identification of MNPs in environmental soil sample. In addition, since particles may have intrinsic fluorescent properties, they can be more easily localized by light microscopy and identified by Raman thus speeding up the analysis.

### **3.08.P-Tu175 From Wastewater Treatment Plants to the Terrestrial Environment: Microplastics in Sewage Sludge Compost, Agricultural Soil, and Earthworms**

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Wastewater treatment plants have been found to be a significant source of microplastics to the terrestrial environment. After wastewater purification, most of the microplastics in the wastewater influent end up into the resulting sewage sludge, which then can be processed further, for example, into compost. The microplastics enter the environment when the compost is applied as a fertilizer to a soil. From the soil, the microplastics can be digested by soil dwelling organisms, like earthworms. The objectives of this study were 1) to develop sample preparation methods for isolating microplastics from sewage sludge compost, soil and earthworm samples, and 2) to investigate the number concentration of microplastics in these samples. Sewage sludge compost, agricultural soil fertilised with sewage sludge compost, control agricultural soil without compost fertilizer and earthworms taken from the same soils were sampled for microplastics. Sample preparation was tailored to each of these specific sample matrices with methods including sieving, filtering (20 µm), organic matter removal and density separation. The prepared sample was then filtered onto an Anodisc filter (pore size 0.2 µm, diameter 25 mm). An imaging Fourier Transformation Infrared microscope (µFTIR; Lumos II, Bruker) was used to identify the microplastics in transmittance mode. Particular effort was put to mitigate sample contamination. Sample preparation and FTIR-analysis were conducted in a separate, synthetic-fibre-free laboratory meant to be used only for microplastic research. Laboratory blank samples were handled similarly with the real samples to assess the sample contamination from the laboratory environment. In this presentation, the number concentration of microplastics in sewage sludge compost, agricultural soils and earthworms are shown and the links between the microplastic sources and sinks are discussed.

**3.08.P-Tu176 Spatial and Vertical Distribution of Microplastic Within the Soil of a Sustainable Urban Drainage System**  
**Max Beaupaire<sup>1</sup>, Rachid Dris<sup>1</sup>, Johnny Gasperi<sup>2</sup>, Marie Christine Gromaire<sup>1</sup>, Romain Tramoy<sup>1</sup> and Bruno Tassin<sup>1</sup>, (1)LEESU, France, (2)Gustave Eiffel University, France**

As urban roads represent a significant aspect of human activity, evaluating their impact as a source of macro-litter and microplastics is necessary to better understand plastic transfer in continental environments. In particular, the microplastic input into soils of sustainable urban drainage systems via urban runoff and their infiltration through soils remain to be assessed. One way to estimate the microplastic input related to road activity and determine the role played by urban runoff in their transport, is to focus on an infiltration ditch on the side of a road.

In this study, the soil of a seepage ditch on the side of a high traffic suburban road in the Parisian region was studied for microplastic content, along with its incoming urban runoff water and outlet water. Soil samples were collected by manually coring at a depth of 35 cm. To assess a potential spatial variation along the seepage ditch, a total of 10 cores were collected. Each core was slightly by depth into 4 samples at [0-5], [5-15], [15-25], [25-35] cm. After collection, microplastics were isolated from the samples using a chemical treatment of organic particles (H<sub>2</sub>O<sub>2</sub>) followed by a density-based removal of minerals (NaI). Finally, samples were deposited on alumine filters and their microplastic content was determined using an automated Fourier Transform Infrared Micro-Spectroscopy mapping analysis (for particles > 25 µm). Preliminary results showed an overall median concentration of 13.4 MP/g of soil, with no significant difference between samples of different depth. Preliminary results on water samples remain to be assessed, though seem to point toward significantly lower microplastic concentrations at the outlet of the ditch than in the urban runoff (inlet), suggesting that the soil retains efficiently microplastics larger than 25 µm.

**3.08.P-Tu177 Exploring the Fate and Impacts of Microplastics in Agricultural Soil: Perspectives from the European Spatial Survey in the PAPILLONS Project**

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Agricultural soils have been highlighted as an important recipient of microplastic (MP). Several sources or pathways of MP to soils have been identified in the literature but the relative importance of these has not yet been quantified. There is a demand to establish the scales of MP pollution in soils and better understand which conditions lead to high levels of pollution. The H2020-funded PAPILLONS project (Plastic in Agricultural Production: Impacts, Lifecycles, and LONG-term Sustainability) will address this knowledge gap by conducting a European spatial survey (ESS) of MP pollution in farmed soils. Sampling has been undertaken in seven countries (Finland, Germany, Norway, Spain, Italy, Greece, and the Czech Republic) representing different biogeographic regions and important areas for European agricultural production. Three main sources of MP to soils were identified: 1) use of agricultural plastics, in particular thin films; 2) application of sewage sludge to land; and 3) use of selected compost products suspected to contain MP. Fields were chosen to provide good coverage of each. Control fields were also sampled, representing MP inputs from additional sources such as atmospheric deposition or littering. The objectives of the ESS are: 1) to establish a baseline for MP concentrations in agricultural soils in Europe; 2) to identify the relative contributions from different MP sources; and 3) to unravel how local or regional agricultural practices can influence levels of MPs in soils.

The ESS represents an ambitious study where several components will be investigated. MPs will be analysed in soils, as well as earthworms collected from different soil depths. Soil samples will also be analysed for macroplastics, plastic additive content and several soil properties. The ESS will also investigate biodiversity in fields subject to potential MP sources and control fields; namely, by assessing communities of earthworms and microorganisms. Finally – to accompany field sampling – farmers were asked to complete a questionnaire to obtain information on practices, behaviours, and perceptions related to plastic pollution in European agriculture.

This poster presents the rationale and design for the ESS in the PAPILLONS project. The findings from this study will provide valuable data about relevant sources of MP to agricultural soils in Europe that can be used to tailor effective solutions for reducing MP pollution.

### **3.08.P-Tu178 Exploring the Fate and Impacts of Microplastics in Agricultural Soil: Perspectives from the Long-Term Field Experiments in Northern, Central, and Southern Europe in the PAPILLONS Project**

**Salla Kristiina Selonen<sup>1</sup>**, Paula E. Redondo<sup>2</sup>, Sylwia Adamczyk<sup>3</sup>, Wulf Amelung<sup>4</sup>, Matty P. Berg<sup>5</sup>, Melanie Braun<sup>4</sup>, Darina Dvorakova<sup>6</sup>, Hannu Fritze<sup>3</sup>, Cornelis A.M. van Gestel<sup>5</sup>, Jari Haimi<sup>7</sup>, Rene Heyse<sup>4</sup>, Jakub Hofman<sup>8</sup>, Rachel Hurley<sup>9</sup>, Sarmite Kernchen<sup>10</sup>, Shin Woong Kim<sup>11</sup>, Christian Laforsch<sup>12</sup>, Martin G.J. Löder<sup>10</sup>, Virtudes Martinez-Hernandez<sup>13</sup>, Raffaella Meffe<sup>13</sup>, Andreu Rico<sup>13</sup>, Johanna Nikama<sup>3</sup>, Matthias Rillig<sup>11</sup>, Vili Saartama<sup>7,14</sup>, Klara Šmidova<sup>8</sup>, Helena Soinne<sup>3</sup>, Jyri Tirroniemi<sup>14</sup>, Aristeidis S Tsagkaris<sup>6</sup>, Sannakajsa Velmalä<sup>3</sup> and Luca Nizzetto<sup>8,9</sup>, (1)Suomen ympäristökeskus (SYKE), Finland, (2)Fundación IMDEA Agua, Alcalá de Henares, Spain, (3)Natural Resources Institute Finland (Luke), Finland, (4)Bonn University, Germany, (5)Amsterdam Institute for Life and Environment (A-LIFE), Vrije University Amsterdam, Netherlands, (6)University of Chemistry and Technology Prague, Czech Republic, (7)University of Jyväskylä, Finland, (8)RECETOX, Masaryk University, Czech Republic, (9)Norwegian Institute for Water Research (NIVA), Norway, (10)University of Bayreuth, Germany, (11)Freie Universität Berlin, Berlin, Germany, (12)University of Bayreuth, Animal Ecology I, Germany, (13)IMDEA Water Institute, Spain, (14)Finnish Environmental Institute (SYKE), Helsinki, Finland

Mulching films are widely used in agriculture and horticulture to facilitate cultivation and to improve the yield. They also have some positive environmental aspects by allowing reductions in the use of irrigation water and plant protection products. However, mulching films have also been identified as a source of plastic additive chemicals and microplastics in agricultural soils.

Biodegradable mulching films have been developed as alternatives to conventional plastics. Biodegradable mulching films are intended to be left in the field and incorporated into the soil after use. Despite the increase in the use of these new materials, their impacts in soil are still poorly known. Moreover, the impacts of microplastics in soil are mostly studied in controlled laboratory conditions, whilst field studies with varying interactions and environmental conditions are scarce.

In the field experiments of the H2020 funded project PAPILLONS (Plastics in agriculture: impacts, lifecycles and long-term sustainability), the fate and impacts of microplastics are studied in real European agricultural environments. The experiments are being carried out in three different countries representing different vegetation and climate zones in Europe: Finland, Germany, and Spain. In each country, the experiment started in the beginning of the growth season in 2022, by introducing two types of microplastics in the study plots at two concentrations and five replicates. The two types of microplastics were produced by grinding pellets that were made of recycled mulching films: one from conventional polyethylene (PE) films and the other from biodegradable films composed of a blend of starch and polybutylene adipate co-terephthalate (starch-PBAT blend). Both experimental concentrations represent the range found in agricultural soils: 0.005% and 0.05% per dry weight of soil. Malted barley grown in the experimental fields of all three countries. Before seeding and at the end of the two growing seasons, (2022 and 2023), soil samples are taken to analyse physicochemical properties of the soil, microplastics, plastic additives, soil invertebrates and microbial community and activity.

Barley growth, stress indicators, and crop quality are also measured.

In this presentation, this holistic field experiment of PAPILLONS project is introduced. The experiment gives a comprehensive picture of the effects and fate of microplastics in real, varying environmental conditions across Europe.

### **3.08.P-Tu179 Characterization of Plastic Contamination in Biogenic Matrices Intended for Reuse in Agriculture**

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The reuse of treated biogenic wastes in agriculture allows the recovery of nutrients, representing an alternative to their disposal, and contributing to the circular economy. However, the presence of plastics in these matrices, as evidenced by various studies, can lead to further plastic input into agricultural soils, thus rising a question on possible consequences for the environment.

Notwithstanding, the current knowledge concerning the presence of plastics in biogenic matrices is very poor. Therefore, the objective of the present study was a quali-quantitative characterization of plastics in different matrices reused in agriculture as: manures, digestate, compost and sewage sludges. Plastics were quantified and characterized in terms of polymer composition, color, shape and size using the Fourier transform Infrared Spectroscopy coupled with an optical microscope ( $\mu$ FT-IR) in Attenuated Total Reflectance (ATR) mode. We measured a variable presence of plastic debris ranging from 0.2 items/g wet weight (w.w.) in manures up to 7 items/g w.w. in sewage sludge. Fibers were the prevalent form and plastics were mostly in the micrometric size. The most abundant polymers were polyester (PEST), polyacrylic rubber, polypropylene (PP) and polyethylene (PE). A different situation has been observed for compost, in which we identified 986 items/g w.w., with a significant increase

compared to the other matrices ( $p < 0.01$ ), mostly made of compostable and biodegradable plastics. The remaining 11% of compost consisted in fragments of PEST and PE. Our study showed the presence of plastics in all investigated matrices, albeit with significant differences in the content among compost and the other matrices. A broad monitoring of plastic particles in such residues, to be coupled with an ecotoxicological assessment, should be promoted to guarantee safe agricultural application.

### **3.08.P-Tu180 A Citizen Science Approach for Identification of Environmental Plastics**

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Terrestrial microplastics (MP) are an emerging environmental challenge. Citizen science is an increasingly important tool for environmental data collection efforts; however, new citizen science approaches are needed to characterize and quantify environmental plastic pollution. As macroplastic waste can further degrade and fragment to the MP size range, identifying micro-, meso-, and macroplastics in the environment can improve MP source tracking. Methods currently used to identify plastic resins require expensive instruments such as Fourier-transform infrared spectroscopy or Raman spectroscopy. In this study, a simple procedure was developed for citizen scientists to identify environmental plastics obtained from various media including water, soil, and sediments. Plastics able to be identified from the method include polystyrene, polyethylene, polypropylene, polyvinyl chloride, nylon, teflon, and polyurethane. The method procedure uses commonly found materials and incorporates three types of tests: density, burning, and solvent tests. The developed procedure was tested by over 100 participants including undergraduate students and community science volunteers. The accuracy of the citizen scientists to identify polyvinyl chloride/teflon was the highest with >95% accuracy. It was determined that the burning test was the most challenging part of the procedure and the lowest percentage of correct answers was observed (65% on average). Environmental macro- and mesoplastics were collected, and were identified using the proposed method with confirmation by atomic force microscopy-based infrared spectroscopy (AFM-IR). Development of an accurate and validated method for identification of environmental plastics can move citizen science efforts focused on plastic wastes beyond bulk MP occurrence to plastic resin identification, which will advance our ability to activate citizen scientist networks to contribute to MP research efforts.

### **3.08.P-Tu181 Bridging the Identification and Quantification of Microplastic Particles in Agricultural Soil Samples**

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Microplastics particles have been detected in the sea, freshwater, land, and air. In addition, plastics have been found to release toxic substances and adsorb pollutants. MP particles have also been experimentally determined in fish and seafood, honey, beer, and salt. To date, there are no established definitions or standardized analytical methods for the detection of microplastic particles. Depending on how the samples were collected, prepared, and analysed, the results could vary by several orders of magnitude. In this study we investigated the feasibility of using the Agilent 8700 Laser Direct Infrared (LDIR) imaging to characterize and identify MP particles in agricultural soil samples.

Soil samples from different depths (0-30 cm, 31-60 cm, 61-90 cm) were taken at nine locations on farmland near Bonn (North Rhine-Westphalia, Germany). All samples went through different purification steps and a density separation and were subsequently filtered through a gold filter (0.2  $\mu$ m, 25 mm). After drying the gold filter, it was fixed on a glass slide for the measurement in the LDIR using a drop of water.

An average of 744 859 MP particles/g soil sample were detected in the 27 soil samples. This corresponds to an average of 838 683 MP particles/g at 0-30 cm, 583 980 MP particles/g at 31-60 cm, and 624 268 MP particles/g at 61-90 cm soil depth, with the major polymers identified being polyethylene (PE), polyamide (PA), and other natural polyamides.

### **3.08.P-Tu182 Method Development for Extraction of Microplastics from Organic Rich Soils**

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Microplastics pollution is widespread in all environmental matrices and considered as a major environmental concern. Identification and quantification of microplastics in environmental matrices has been challenging, especially the contamination in terrestrial system is poorly known given due to the complexity of the soil types and lack of standard approach for microplastic extraction. This study presents a comparison of microplastic recovery from different types of soil comprising of a range of complexity particularly with organic matter. Here we used 3 different types of soil with organic matter Lufa 2.0 soil, compost, and topsoil. All the soils were air dried and sieved with 2.0 mm mesh to remove large debris. Soils were amended with 10 particles each of 9 different microplastics comprising different polymers, shapes, sizes, and colours. Fibres were stained with Nile Red prior spiking for better visualization. Organic matter was removed from the soils using an overnight digestion in Fenton's reagent (60% H<sub>2</sub>O<sub>2</sub> and FeSO<sub>4</sub>). After the digestion, microplastics were separated from the digested soil by flotation in a ZnCl<sub>2</sub> solution. Samples were centrifuged, and then filtered through a nylon filter. Particles were observed using fluorescence light microscopy and the recovery in each soil type was determined. Microplastic separation performed better in low organic soil with higher recovery. Soils rich in organic matter requires improved protocols involving further purification to gain better recoveries.

### 3.08.P-Tu183 Status quo of Soil Sampling for Microplastic Analysis (SOSMA)

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Determination of microplastics (MP) in soils has gained enhanced scientific attention in the last decade. This talk addresses current state of the art in terms of sampling and sample preparation to derive information on MP content in soils. We conducted a systematic literature review by in-depth screening of more than 100 articles that focused to account for MP burden in soils (particle counts or masses). Most of these studies (63%) focused on known emission pathways with discrete study sites, whereas 37% targeted to delineate MP background contents due to fluvial, marine, or aeolian MP deposition with de-localized MP emission sources. Thereby, 93% of the studies investigated the top 30 cm, which highlights the knowledge gap of MP abundance in the subsoil. Even though soil was the target medium, soil organic matter content, pH, and texture were measured only in 20% of the studies. However, such basic soil parameters need to be considered in particular to evaluate analytical results for MP, e.g., of recovery tests. In terms of spatial distribution of soil samples, random sampling was applied more frequently than sampling of transects and raster sampling. In rare examples, the composite sample taken in the field was based upon a large representative elementary volume (REV) of several 100 kg that was reduced afterwards by quartering and mass reduction. However, the majority of studies followed common procedures where the mass of the composite sample ( $2.150 \pm 1.150$  g) constitutes merging of a distinct number of single samples ( $n = 5.1 \pm 4.6$ ). Larger REV's seem promising and might be an aspect to facilitate reproducible and representative analytical results, which needs to be validated for emission-specific MP pathways into soils. In conclusion, the systematic review revealed an incoherence in soil sampling and sample preparation within analytic protocols, which renders a comparison of results challenging.

### 3.08.P-Tu184 iMulch - An Investigation of the Influence of Polymers on a Terrestrial Ecosystem Using the Example of Mulch Films used in Agriculture

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Plastics enter soils directly and indirectly. Mulch films represent an exemplary use of plastics on soils that can lead to direct plastic input. Mulch films are used in agriculture to regulate temperature and moisture in the soil, prevent weed growth and soil erosion during heavy rainfall, and protect crops from pests and predators. It is to be expected that in the course of climate change and the intended reduction in the use of pesticides, there will be a growing use of mulch films in agriculture. Whether the use of mulch films on agricultural land can have a negative effect on the soil was analysed from different perspectives in this project.

- A method for the analysis of microplastics in soil samples was developed for Raman spectroscopy and Thermo extraction desorption Gas chromatography mass spectrometry (TED-GCMS).
- Different soils were analysed for the plastic content and the type of agriculture was correlated to the results
- The aging of the mulch film was analysed in laboratory and mesocosm soil test and additionally in a laboratory sewage treatment plant.
- The transport and fate of the mulch-polymers in soils using <sup>14</sup>C marked polymers in outdoor lysimeter experiments was investigated
- Experiments examining effects of the mulch fragments on organism were conducted.
- The “upcycling” of mulch films by bacteria was investigated.
- A life cycle assessment of conventional and biodegradable agricultural mulch films was conducted.
- An avoidance and substitution strategy was derived from the results with the aim of reducing plastic film fragments in the environment.

This presentation will give an overview of the results of this project.

### 3.08.P-Tu185 Tyre and Road Wear Microplastic Identification and Transport in the Urban Environment

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Car tyre, brake, and road surface wear materials have been identified as major sources of microplastic pollution in the environment, in particular, in urban areas. Although they have been recognised as significant contributors of microplastic, due to analytical challenges only few studies to date include tyre and road wear particles in quantitative assessments of microplastic pollution. Therefore, we are facing a significant knowledge gap as to how these particles behave in the environment, bearing the risk of significant undetected environmental impacts now, and in the future. As such, this study aims to improve methods for the quantitative analyses of tyre and road wear particles found in environmental samples, in addition to investigating their initial transport pathways away from their site of generation in the urban environment.

Here we present the sampling methodologies developed by us for the collection of atmospheric deposition samples, inclusive of tyre and road wear particles from a major urban road in Birmingham, UK, together with the laboratory methods used to prepare



samples for analyses of particles using Pyrolysis GC MS and microscopy. Atmospheric deposition samples were collected for different intensities of traffic flow and across a range of different precipitation events in order to understand how individual environmental factors affect the transport of tyre and road wear particles to the immediate surrounding roadside environment. This ongoing sampling campaign, is currently extended by the sampling of airborne particles, and particles from the direct road runoff, to understand the real time contribution of tyre and road wear particles to each transport route. Pyrolysis GC MS methods for the identification of tyre and road wear particles are being trialled for the identification of 'real world' tyre and road wear particles from environmental samples collected, with the aim to improve marker compounds for particle quantification. We hypothesise that traffic volume and the specific type of precipitation event, including its antecedent conditions, will have the most significant impact on tyre and road wear particle load in the environmental samples collected.

### **3.08.P-Tu186 Microplastics in Total Atmospheric Fallout: From Lockdown to Business-as-Usual**

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Since the earliest studies on microplastic (MP) pollution in the atmospheric compartment in 2015, a few dozen studies have been published on the subject. Over that period, analytical methods and targeted MPs have evolved. Several factors are suspected by authors to affect MP atmospheric deposition, human activity and precipitation being the leading ones.

In this work, total atmospheric fallout from an urban campus (site A) was sampled over three continuous monitoring campaigns of 4 to 6 months each. Campaigns in site A occurred during the French national lockdown of spring 2020 as human activity was drastically reduced there, and in spring 2021. The monitoring campaign in site B occurred in spring 2021, simultaneously to site A. In all campaigns, precipitations were followed to adjust sampling accordingly.

All samples underwent a density separation followed by a chemical treatment, before they were analysed using an automated  $\mu$ FTIR mapping analysis using a Nicolet iN10 by Thermo Scientific. MPs were identified down to a size of 25  $\mu$ m, cutoff point determined by the  $\mu$ FTIR detectors.

When pooling all results together, overall median deposition rates of 18.3 MP/m<sup>2</sup>/d were found. The lowest deposition rates were recorded during the national lockdown monitoring campaign in site A, with a median of 5.3 MP/m<sup>2</sup>/d compared to 26 MP/m<sup>2</sup>/d in the same site during normal periods of activity. Based on the rain events and precipitation rates, no clear effect of rain events or accumulated rainfall on microplastic deposition was measured, suggesting the dominant effect observed here was local human activity.

### **3.08.P-Tu187 Marking Stripes in Durable Woven Mulch Films are Their Major Source of Microplastics**

**Sarmite Kernchen<sup>1</sup>, Martin G.J. Löder<sup>1</sup> and Christian Laforsch<sup>2</sup>,** (1)University of Bayreuth, Germany, (2)University of Bayreuth, Animal Ecology I, Germany

Applying mulch films to fields is a common agricultural practice that increases the quality and yield of cultivated crops. Mulch films act as a shield that protects plants from weeds, insects, and pests, keeps moisture in the soil, protects roots from direct sunlight, prevents soil erosion, and keeps the soil fertile. Recently, however, the application of mulch films to fields has been shown to contaminate soil with macro/micro/nanoplastic particles that can negatively impact soil ecosystems. As impacts are expected to be proportional to soil microplastic (MP) concentrations, it is critical to determine the level of MP pollution in agricultural soils and practices to assess future scenarios and provide guidance for sustainable agricultural policies. Chemical composition, application duration, and end-of-life practices of mulch films are the most important factors affecting the rate of film degradation and subsequent soil contamination with MP.

Next to mulch films made of polyethylene (PE) or biodegradable plastics, durable woven mulch films are also available. While films from PE or biodegradable plastics are not meant for a long-term application, durable woven polypropylene (PP) mulch films promise a multiyear stability. However, these durable mulch films have not yet been studied in detail for their potential as a source of MP. Therefore, in this study, we characterized and quantified MP in German agricultural fields where black woven PP mulch films with green PP marking stripes were applied over a 12-year period. MP particles were extracted from soil samples by density separation, purified using an oxidative-enzymatic protocol and analyzed by micro-Fourier transform infrared spectroscopy, which provided information on the chemical composition, shape, size, and color of the particles. The results showed that PP fragments, apparently derived from the application of mulch films, significantly contaminated the soil. The average MP content per kilogram of soil was 5005 $\pm$ 1298 particles, most of which came from a weathered mulch film. Interestingly, the majority of MP particles came from the green marker strips rather than the black fabric. These results suggest a difference in UV stabilizer content between black and green PP material, which needs to be verified by in-depth chemical analysis. Our work highlights the fact that different components of durable woven mulch films may play different roles as significant sources of MP entering agricultural soils.

### **3.08.P-Tu188 Microplastic Contamination in an Urban River in Germany – Investigating and Tackling the Challenge of Organic-Rich Matrix**

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The ubiquitous distribution of microplastics globally has attracted much attention and their contamination in rivers is noted as a substantial environmental problem. Aside from being considered as a transport pathway of land-derived plastic to the ocean, rivers serve as both (temporary) sinks for land-based plastic pollution, as well as future sources due to remobilization. However, the research thus far mainly focuses on larger rivers, while the situation in smaller rivers is poorly understood.

Freshwater samples are often rich in natural particles, complicating microplastic analysis using micro-Fourier-transform infrared spectroscopy ( $\mu$ -FTIR). The extraction of microplastics from the samples while preserving them is challenging, especially the removal of suspended organic matter particles, which mainly have a density in the same range as most common plastics and are also not possible to remove without chemical digestion.

In the present study, the dynamics of microplastic contamination of an urban subcatchment, the Parthe river, is investigated. The river is in southeastern Germany and passes through several towns mainly characterized by urban and agricultural land use. In this work, a fractionated filtration sampling device was developed to collect the suspended matter. Samples were collected from two different land use sites once a month for one year to cover all seasons. Since the samples from the Parthe River are rich in suspended organic matter, mainly plant debris, existing (ligno)cellulosic digestion/oxidation methods were tested and optimized. By analyzing the mass reduction, NaOCl oxidation showed the highest efficiency of organic matter removal eliminating up to 97 % weight of the sample matrix. To accomplish microplastic concentration examination, suspended matter samples were purified by combining the optimized protocol including NaOCl treatment and density separation, followed by determining microplastic concentration using  $\mu$ -FTIR. According to preliminary results, 53 and 57 particles/m<sup>3</sup> of microplastics were found in a sample fraction of 100 - 500  $\mu$ m from an urban and agricultural area, respectively. Microplastics in the Parthe River samples are predominantly polypropylene (PP) and polyethylene (PE). Our findings will provide a better understanding of the dynamics of microplastic contamination in the urban river and will further identify sources of microplastic contamination in the rural as well as the urban section of the Parthe catchment.

### 3.08.P-Tu189 Effects of Conventional and Biodegradable Microplastic on Seed Germination and Plant Growth of Food Crops

**Laura Zantis<sup>1</sup>, Anne Wilts<sup>1</sup>, Sannakajsa Velmala<sup>2</sup>, Sylwia Adamczyk<sup>2</sup>, Martina G. Vijver<sup>3</sup>, Willie Peijnenburg<sup>4</sup> and Thijs Bosker<sup>3</sup>,** (1)Leiden University, Institute of Environmental Sciences, Netherlands, (2)Natural Resources Institute Finland (Luke), Finland, (3)Leiden University, Leiden, Netherlands, (4)National Institute for Public Health and the Environment (RIVM), Netherlands Nano- and microplastic (<100 nm and <5 mm, respectively) pollution is becoming increasingly persistent in all ecosystems and is considered an emerging threat to biodiversity and ecosystem functioning. One major concern is agricultural lands, which have been identified as long-term microplastic sinks. As a result, there is now a push toward developing bioplastics, which can be (partly) broken down after entering the environment. Yet, limited research has been done to compare the impacts of microplastics and bioplastics on terrestrial crop plants. This study aims to examine and compare the effects of artificially weathered conventional polyethylene (PE) and starch-based biodegradable polybutylene adipate terephthalate (PBAT) on four common food crops, including lettuce (*Lactuca sativa* L.), carrot (*Daucus carota*), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare*), through two complementary experiments. We investigated the effects of environmentally relevant low, medium, and high (0.01%, 0.1%, 1% w/w) concentrations of weathered PE and PBAT particles on seed germination (acute toxicity: Petri-dish experiment) and plant growth (chronic toxicity: pot-plant experiment), respectively. Our results show adverse effects of both PE and PBAT on crops. When acutely exposed during germination, PE significantly accelerated the germination rate of carrot seeds by two days ( $p < 0.05$ ). In addition, both PE and PBAT decreased the root length of lettuce and wheat by up to 36% and 53% respectively ( $p < 0.05$ ). Contrary, root length ( $p < 0.0001$ ), shoot length ( $p < 0.05$ ), and seedling biomass ( $p < 0.05$ ) of carrot were increased with up to 86% when exposed to PE and PBAT, respectively. In the pot-plant experiment, PBAT significantly delayed the germination rate of barley ( $p < 0.05$ ), and additionally decreased the shoot biomass by 16% after 14 days ( $p < 0.05$ ). No significant effects of PE and PBAT were recorded on the seed germination of lettuce. Overall, these results show that the early development of seedlings is mainly impacted, but over time plants might recover mildly. Moreover, biodegradable plastic was more toxic than the conventional plastic highlighting the need to further investigate the effects of micro- and bioplastics on crop plants.

### 3.08.P-Tu190 Long-Term Effects of Agricultural Microplastics on Earthworm *Eisenia Andrei*; Comparison of Conventional and Biodegradable Plastics

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Use of plastics is common in modern agriculture, but degradation of plastics in soil is a slow process. Biodegradable plastics have been proposed as a solution for this problem, but little is known about possible long-term effects of plastics, either biodegradable or conventional, on soil organisms. The aim of our study is to find out the possible effects of microplastics from conventional and biodegradable mulching film material on the earthworm *Eisenia andrei*.

We studied two types of microplastics produced by grinding pellets made of recycled mulching films, one from conventional polyethylene (PE) films and the other from biodegradable films composed of starch and polybutylene adipate co-terephthalate (starch-PBAT). We exposed earthworms to these two microplastic types in concentrations of 0%, 0.005%, 0.05%, 0.1%, 0.5%, 1%, and 5% of dry mass. The experiments started with standard reproduction tests with earthworms of parental (P) generation (ISO 11268-2). After the standard tests, we selected 13 juveniles from each test jar for a new set of tests to study the impacts of long-term microplastic exposure on the first offspring generation (F1). We followed the growth and the number of mature

individuals every four weeks. In addition to survival, growth, and reproduction, the uptake of microplastics and plastic additives as well as immunological biomarkers in earthworms will be analyzed.

The experiments are still running, and statistical analyses have not been performed yet. The preliminary data suggest that the microplastics have not affected earthworm survival, but the presence of PE in the soil decreased the growth in F1 generation. In contrast, the biodegradable plastic seems to increase the growth of the earthworms in F1 generation. The earthworms in the highest PE concentrations also matured later.

### **3.08.P-Tu191 The Effect of Soil Plastic Contamination on Agricultural Production**

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Agricultural soils act as long-term sinks for plastic contaminants. Plastics enter agricultural soil through fertilisation with sewage sludge and biowaste compost, which are high in microplastics, or through fragmentation of larger plastics, such as plastic mulching. Sewage sludge is used widely in Europe as a fertiliser. Through this route, it is estimated that between 63 000 and 430 000 tonnes of microplastics are annually deposited on European farmlands. Reports have shown that microplastics affect soil microorganisms, nutrient cycling, and plant growth; however, the effects they exhibit depend on their size, concentration, shape, and composition, therefore the overall effect is extremely hard to determine. This study investigated the effect of 'cocktail' plastic contamination on nitrogen cycling in soil and tomato production. Concentrations, shapes, and compositions of plastics were chosen to resemble plastic contamination found in agricultural soil, fertilised with biowaste compost for 20 years.

The results from a pot experiment, where tomatoes were grown in the presence of meso- and microplastics, will be presented. We investigated the effect of plastic contamination on plant biomass, tomato fruit ripening, and the biochemical composition of tomato fruits. Additionally, we investigated the effect of plastic contamination accumulated in the lower layer of the substrate versus homogeneously distributed plastic contamination. This is because we found that in ploughed fields, most of the plastic contamination is found in the 10-20 cm layer of the soil. We hypothesise that plant biomass will be bigger in the plastic-contaminated substrate due to increased substrate aeration and macroporosity, whereas no effect will be observed in the biochemical composition of tomato fruits. Furthermore, we will present the results from a microcosm experiment, exploring the effects of plastic contamination on nitrate concentration and microbial community composition in soil and organic-rich substrate. We hypothesise that nitrate production will be lower in the plastic-contaminated soils and substrate; however, if contaminated with biodegradable plastics, the nitrate production might be higher.

The results of this study will contribute towards a better understanding of the effects of 'cocktail' plastic contamination in agricultural soil, specifically on nutrient cycling and crop production, and provide an insight into a potential effect on future food security.

### **3.08.P-Tu192 Biodegradable Plastics – An Improvement or a Hazard to Soil Organisms? Assessment of Effects of Microplastics and Leachates on Earthworms, Plants, and Soil Respiration**

**Luís André Mendes**<sup>1</sup>, **Jorge Domínguez**<sup>1</sup> and **Ricardo Beiras**<sup>2</sup>, (1)Universidade de Vigo, Spain, (2)University of Vigo, Spain

Plastic pollution has been the subject of immense news coverage and concern, raising issues regarding environmental safety and long-term impact on the ecosystems. In response, several manufacturing companies have produced biodegradable goods, presented as a safer-by-design solution for the environment, relative to most common fossil-based plastics. However, is this true for soil ecosystems?

In the present study, we propose assessing the response of three different soil components, i.e., invertebrates, plants and microbial respiration, to biodegradable and fossil-based plastics used at environmentally relevant concentrations on 3: following OECD and standard test guidelines. We used powdered plastic samples (particle size <250 µm) and leachates obtained from the same materials, to evaluate whether the source of toxicity was the microplastic itself or the associated additives.

OECD test no. 222 was used to measure survival and reproduction of the model earthworm species *Eisenia andrei* exposed to plastic-contaminated soil for 28 and 56 days. OECD no. 208 guideline was adapted to assess the germination index (GI), relative root growth (RRG), relative seed germination (RSG) and relative shoot growth (RShG) of the garden cress *Lepidium sativum* grown for 7 days in the contaminated soil extracts. Finally, OECD no. 216 guideline was adapted to measure the levels of basal respiration of soil exposed to similar conditions as the earthworms.

Survival of *E. andrei* was not modulated by plastic contaminated soil; however, earthworm reproduction was altered, with significantly fewer cocoons and juveniles produced in soil contaminated with either the powdered plastic or the leachate. Soil basal respiration was significantly increased by low concentrations of FBP leachates. As for the RShG of *L. sativum* was increased by exposure to the leachate but not by powder at similar low concentrations. However, FBP powder induced an increase in GI.

The findings indicate that the use of biodegradable plastics does not yield any improvement regarding negative effects on soil. More importantly, although the earthworm reproduction was directly affected, low concentrations of plastic-based leachate can

alter the soil microbial activity in the form of microbial respiration, potentiating plant growth, with consequences on the soil ecosystem.

### 3.08.P-Tu193 Effects of Agricultural Microplastics on Two Soil Invertebrates: Comparison of Nondegradable and Biodegradable Oil-Based Plastics

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Agricultural plastics are considered one of the most important inputs of microplastics into the soil environment. Therefore, terrestrial invertebrates are exposed to various types of microplastics. Biodegradable plastic mulch films have emerged as a sustainable alternative to conventional plastic mulch films in agriculture, promising to reduce soil contamination with plastic residues through in situ biodegradation. However, the biodegradation of such plastics under natural conditions may differ from standardized tests, so they may also become a source of microplastics in terrestrial ecosystems. Currently, there is very few soil ecotoxicity data available for biodegradable plastics. In this context, the aim of this study was to compare the effects of oil-based, non-biodegradable low-density polyethylene (LLDPE) and starch-based, biodegradable butylene adipate-co-terephthalate (PBAT) microplastics cryo-milled from mulch films used in agriculture. The medium size of both types of microplastics was ~500 µm. We investigated the effects on terrestrial crustacean woodlice *Porcellio scaber* and mealworm larvae *Tenebrio molitor* through soil exposure. Woodlice were exposed for 2 weeks following the survival, growth and immune response. Mealworms were exposed for 8 weeks, following the survival, growth, moult, and development (emergence of pupae and adults). The results showed that none of the investigated microplastics affected the survival and growth of woodlice and mealworms, but the immune response of woodlice was induced and moult of mealworms was affected by both types of microplastics. Thus, no clear differences between effects of LLDPE and PBAT were observed. This study suggests that further comparative studies with microplastics derived from non-degradable and biodegradable plastics are of interest.

### 3.08.P-Tu194 Investigating the Toxicity of Biodegradable Plastic Textile Fibres on the Earthworm *Eisenia fetida*

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Biodegradable polymers have been proposed as part of the solution to combat rising quantities of plastic in the environment, and have increased in their production over the last decade. Biodegradable plastics have wide application, including in textiles and wet wipes, where fibres may become emitted to the environment through wastewater discharge and the application of biosolids onto agricultural land. Once in the environment, the rate at which biodegradation occurs is poorly evaluated. Additionally, there are notable gaps in assessing the ecotoxicological implications of biodegradable polymers on organisms and ecosystem functioning. This study examined the toxicity of two fibrous biodegradable polymers derived from textiles (viscose and lyocell), compared to a conventional polymer (polyester) on the earthworm, *Eisenia fetida*. Initial 72-hour toxicity tests on damp filter paper were undertaken using a concentration series of fibres (10,000 – 100 fibres/ml; 125 – 300 µm in length), following the OECD method (OECD 207) to establish lethal thresholds. Subsequently, 28-day chronic exposures were carried out for each polymer at a concentration of 100 mg fibres kg<sup>-1</sup> dw of Lufa 2.2 soil. Earthworms were sampled after 7 and 28 days, recording mortality and mass, along with assessing fecundity and performing behavioural, biochemical and histological analysis. Results indicated that biodegradable fibres induced a similar mortality as polyester over 72-hours (LC<sub>20</sub>: 0.0308 mg/ml lyocell; 0.0157 mg/ml viscose; 0.0090 mg/ml polyester). Research to establish sublethal effects of biodegradable fibres following the 28-day exposures are ongoing and will be shared during this conference. The results of this work will contribute toward data deficiencies regarding the ecotoxicological implications of biodegradable fibres, which are required for the development of ecological risk assessments.

### 3.08.P-Tu195 The Impact of Exposure and Uptake of Nanoplastics on Lettuce Growth and Development

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Commercial horticultural production practices often use plastics to improve crop quality and yield, which can consequently introduce plastics directly into the soil. The weathering of plastic can cause fragmentation from macro- (> 5mm), to micro- (1 µm – 5 mm) and nanoplastics (< 1000 nm). These particles have the potential to alter soil properties and influence the growth of crops. Here, we aim to investigate the effects of spherical nanopolystyrene particles ~ 20 nm diameter (nPS) on lettuce (*Lactuca sativa*) grown in a 'hydroponic system'. The nPS were synthesised using an emulsion polymerisation and characterised with a variety of techniques. Lettuce seeds were pregerminated then exposed to a range of nPS concentrations (0, 0.01, 0.5, 500, and 1000 mg/L nPS in ¼ strength Hoagland's plant nutrient) for 28 days, changing the nutrient mix every seven days. Preliminary results showed that, in comparison to the negative control, all spiked concentrations had a reduction in plant growth parameters (no. leaves, widest and longest leaf length, total leaf area, and fresh aerial plant biomass). Spikes of 1000 mg/L nPS resulted in coagulation of nanopolystyrene in Hoagland's plant nutrient, resulting in smaller plants than the control, but larger plants than 500 mg/L, likely due to the coagulation causing reduced bioavailability of nanopolystyrene. A germination study was also performed using the same exposure concentrations; however, no statistical difference in root or shoot emergence rate were observed. The preliminary results of this study suggest that nanopolystyrene over a range of concentrations can have a negative impact on lettuce development. However, the mechanism of action is not yet understood. Future research will be performed to

determine if lettuce can uptake and translocate nanoplastics. If confirmed, plants could be a potential input of nanoplastics into the food system; and this research can be used to inform industrial practices and new crop cultivation design.

### **3.08.P-Tu196 Influence of Different Plastic Polymers to the Terrestrial Snail *Cantareus aspersus* During a Life Cycle Experiment**

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Plastics accumulate in the environment and can be fragmented, leading to the formation of small plastic fragments (<5mm = microplastics, MPs) which can diffuse and accumulate in various ecosystem compartments. While much work on soil invertebrates has focused on short and acute exposure, little work has been done on the chronic effects of these MPs. Hence, the objective of our study was to identify and measure the response of the terrestrial snail *Cantareus aspersus* to the exposure to different MPs through a life cycle experiment.

Juvenile snails (one month old,  $1.36 \pm 0.2$  g) were exposed in triplicate in glass containers to a range of concentrations (0, 0.1, 1, and 10 % w/w in food) of polyethylene (PE), polyamide (PA), and polystyrene (PS) particles, during a 4 month-experiment. The responses monitored were survival, growth, sexual maturation, fecundity, and fertility. Data were described using logistic models to consider the temporal trends of the measured responses over the exposure duration.

The results show a dose response effect of MP exposure for growth and sexual maturity, with a growth stimulation at 0.1% MP and a growth inhibition at 1 and 10% MP. An earlier sexual maturation was observed at 0.1% MP and a marginal effect of PE at 1%. Fecundity was affected with a polymer effect, especially by PA, with less eggs laid at 1% and 10% and earlier egg laying at 0.1% and 10%. Fertility also showed a polymer effect with earlier hatchings in PA groups, while a contrasted pattern was observed for PS (delayed hatching at 0.1%, earlier at 10%) and PE (earlier hatching at 1%, delayed at 10%). Mortality showed a polymer effect, with no difference in the 0.1% groups, but with an increasing mortality in the controls accordingly to the exposure concentration, when the mortality remains around 4% for MPs-exposed snails.

In this experiment, the snails could potentially be influenced either by the physical effects (for growth), and by chemical effects of the MPs or their additives (for sexual maturity, reproduction, mortality). An additive related endocrine disruption could here disturb both sexual maturity and lower reproduction traits, influencing the energy reserve of the snails and ultimately leading to an increased survival of the animal. This work highlights the relevance of taking together a wide range of biological endpoints considering different aspects of the life cycle to decipher subtle physiological effects of MPs on soil invertebrates.

### **3.08.P-Tu197 Does the Co-Existence of Biodegradable Microplastics and Cadmium Affect the Behaviours of Earthworms Differently from Non-biodegradable Microplastics and Cadmium?**

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Biodegradable polylactic acid (PLA) has been developed to replace non-biodegradable conventional polyethylene (PE), addressing environmental plastic pollution, particularly in the use of arable mulches. Furthermore, fluctuating temperatures and UV radiation can lead to fragmentation of plastic sheeting and the formation of microplastics (MPs) and MPs providing the opportunity to interact with other common contaminants such as cadmium (Cd), which might pose risks to soil organisms. Laboratory exposures, using earthworms as a key soil organism suggest that the combined effects of non-biodegradable MPs and Cd in soil can lead to synergistic toxic effects. Moreover, a differential performance for maize and arbuscular mycorrhizal fungi (AMF) co-exposed to Cd and either non-biodegradable PE or biodegradable PLA and Cd in soil has been detected, but comparatively little is known about the effects of metal-contaminated biodegradable MPs on earthworms. Given this large knowledge gap, it is important to consider the possible interaction between non-essential metal Cd and biodegradable MPs when evaluating the risk that both pose to terrestrial organisms and compared to non-biodegradable MPs-metal interactions. Therefore, experiments to test the single and combined effects of MPs and Cd on the response of earthworms, both biodegradable and non-biodegradable MPs are warranted. We carried out avoidance and exposure tests to investigate whether the co-existence of biodegradable microplastics and cadmium affected the behaviour of earthworms differently from non-biodegradable microplastics and cadmium. In our avoidance tests, earthworms were exposed to 0.3 wt % MPs and / or 0, 0.1, 1.0, and 10 mg / kg Cd. We found a significant difference ( $P < 0.05$ , Holm-Sidak pairwise multiple comparison) in earthworms avoidance between the 0.3 wt % of biodegradable PLA and non-biodegradable PE as well as between all 100 mg / kg Cd treatments and other Cd concentrations treatments (0, 0.1, 1.0, and 10 mg/kg). As for our exposure experiments, earthworms were exposed to 0, 0.1, 0.3 and 3.0 wt % PLA MPs and 0, 1.0, 5.0, 10, 15, 30, 50 and 100 mg/kg Cd both as separate MPs and Cd treatments and in combination. We detected that there was no significant difference in earthworms weight change between all PLA concentrations ( $P = 0.174$ ), and all Cd levels ( $P = 0.797$ ).

### **3.08.P-Tu198 Extent and Impact of Microplastics on Availability of Soil Nutrients: A Trade-off Assessment**

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Ever since the invention of plastics, Microplastic (MPs) pieces are spread ubiquitously, however previous studies mainly had focused on pollution in aquatic systems. More recently, research is focusing on the fate and impact of microplastics on terrestrial ecosystems. In this connection, urban and agricultural soils are an important long-term sink for microplastics. Microplastics may enter the soil through application of sewage sludge and compost, irrigation, plastic mulching, littering, as well as atmospheric

deposition. Microplastics in soil undergo translocation, erosion, degradation, and leach to groundwater, before subsequently taken up by plants and get transferred along the food chain.

While water treatment plants effectively remove microplastics from influent water, microplastics get concentrated in the sludge. This sewage is subsequently spread in agricultural soils for crop production. On the positive side, sewage sludge can supply a large part of the nitrogen or phosphorus that most crops need. It's also a good source of organic matter that can improve the water-retaining capacity and structure of soil. On the other hand, sludge often contains potentially toxic elements, such as heavy metals, pathogens, antibiotics, as well as microplastics. Once in soil, microplastics could induce changes in soil fertility and also pose a potential threat to plant performance and crop productivity.

The objective of this study is to review the extent of microplastics added from the sludge on agricultural land, the potential impacts of sludge amendment on nutrient availability and soil biota. The current study will analyse the data about sludge application and nutrients levels from farmland in central UK and relate these levels in soil with amount of added microplastics.

### **3.08.P-Tu199 Exploring the Fate and Impacts of Microplastics in Agricultural Soil: Perspectives from the Single-species Testing in the PAPILLONS Project**

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Agricultural plastics are considered one of the most important inputs of microplastics into the soil environment. EU Horizon 2020 project PAPILLONS aims to explore various aspects of micro and nanoplastics (MNP) resulting from the use of agricultural plastics, such as sources, environmental fate and ecological effects in agricultural soils. Within the latter, the special focus is to investigate the effects on terrestrial invertebrates. For this purpose, we designed a single-species testing approach using an array of organisms from different taxonomic groups (insects, crustaceans, annelids, nematodes): earthworms (*Eisenia andrei*), springtails (*Sinella curviseta*; *Ceratophysella denticulata*, *Folsomia candida*, *Heteromurus nitidus*), enchytraeids (*Enchytraeus crypticus*, *E. albidus*), nematodes (*Caenorhabditis elegans*), woodlice (*Porcellio scaber*), mealworms (*Tenebrio molitor*) and ants (*Lasius niger*). In this presentation, we will first briefly introduce the test set-up with each of the test organism focusing on the long-term exposure and endpoints that demonstrate chronic effects (growth, reproduction, development, moult). Where possible, multigenerational effects were followed. In the second part, we will demonstrate the approach to soil ecotoxicity testing of MNP focusing on the following relevant issues: 1) selection of relevant agricultural microplastics, 2) selection of relevant exposure concentrations, 3) measures taken to ensure the homogeneity of MNP distribution in test soil, 4) MNP and plastic additive analysis in soil and organisms, and 5) monitoring of soil physico-chemical properties in soil during the experiments. We will show some examples of how these issues have been considered through a case study of two types of microplastics produced by grinding pellets from recycled mulch film: one from conventional polyethylene (LLDPE) films and the other from biodegradable films composed of starch and polybutylene adipate co-terephthalate (starch-PBAT).

### **3.08.P-Tu200 Effects of Nano- & Microplastics on Terrestrial Plants are Ubiquitous and Widespread: A Systematic Review**

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Over the last years there has been significant research on the presence and effects of plastics in terrestrial systems. Here we summarize current research findings on the effects of nano- and microplastics (NMPs) on terrestrial plants, with the aim to determine patterns of response and sensitive endpoints. We conducted a systematic review (based on 78 studies) on the effects of NMPs on germination, plant growth and biochemical biomarkers. The majority of studies to date have used pristine polystyrene or polyethylene particles, either in a hydroponic or pot-plant setup. Our results show that effects on plants are widespread. We noted similar responses among plant species, and between monocots and dicots to NMPs, except for consistent lower germination in dicots due to NMPs. Our results also highlight variation in sensitivity of endpoints to NMPs. During early development, germination and root growth are more strongly affected compared to shoot growth. NMPs induced similar adverse growth effects on plant biomass and length in the most tested plant species (lettuce, wheat, corn, and rice) irrespective of the polymer type and size used. Moreover, biomarker responses were consistent across species; chlorophyll levels were commonly negatively affected, while stress indicators (e.g., ROS or free radicals) and stress respondents (e.g., antioxidant enzymes) were consistently upregulated. Importantly, effects were commonly observed at environmentally relevant concentrations for most endpoints. These findings clearly indicate that NMPs have a wide ranging and ubiquitous impacts affecting plant performance. Importantly, most studies have been conducted under highly controlled conditions and with pristine plastics, raising the urgent need to test under more environmentally realistic condition to ensure the lab-based studies can be extrapolated to the field.

### 3.08.P-Tu201 Effects of Microplastic on a Non-target Organism: Cellular Effects on the Lichen

*Gintare Sujetoviene and Diana Miškelytė, Vytautas Magnus University, Lithuania*

Although plastic pollution is most visible in the environment of anthropogenic sites, there is increasing evidence of microplastics entering the remote environment. As this pollution is becoming an increasingly global issue, lichens as one of the well-known biomonitors of pollution may be useful, although to the best of our knowledge there are no studies on the effects of microplastics at cellular level. The aim of the study was to evaluate the toxic effects of polypropylene (PVC) on viability of terrestrial biota (non-target organism) - the lichen *Ramalina fastigiata*. Thallus of lichen were incubated with significant environmental microplastic concentrations and changes in the photosynthetic apparatus (chlorophyll fluorescence, reduction of chlorophyll a content) and permeability of cell membranes were determined. The study shows that exposure at low ( $\mu\text{g L}^{-1}$ ) microplastic concentrations (within the range of concentrations detected in the environment), induce disturbances in the physiological state of the lichen *R. fastigiata*. In addition, the preliminary results of this work highlight the potential negative impacts of uncontrolled use of microplastics on the components of the environment.

### 3.08.P-Tu202 Microplastic Distribution in Sewage Sludge Amended Soil - The Advantage of Complementary Uses of Py-GC/MS and micro-FTIR Imaging

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Sewage sludge application to agricultural land has been associated with elevated microplastic (MP) loads on soil. Although laboratory studies suggest that microplastics may be transported to deeper soil layers by bioturbation and percolation in macropores, these transport processes have hardly been assessed in field trials yet. There is furthermore a strong divide between studies, either applying spectroscopic methods focusing on particle counts and properties, and thermoanalytical techniques which primarily yield total polymer masses. Field studies analysing both the mass distribution of MPs in soil and particle shapes and sizes are still scarce but needed to better understand their distribution in soil.

We analysed soil core samples along 90 cm depth profiles from agricultural long-term field studies in Sweden to which sewage sludge was applied since 1996. First, we used solvent-based pyrolysis gas chromatography – mass spectrometry (Py-GC/MS) to identify samples of interest. Samples with exceptionally high MP concentrations were subsequently analysed with micro-Fourier Transform Infrared spectroscopy (micro-FTIR) imaging for characterizing sizes, polymer type, and shape associated parameters. The highest MP mass concentrations were found in and directly below the plough layer, successively decreasing with depth, but still detectable at 40 cm depth. We inferred that the observed decreasing mass concentration with depth is probably a result of larger MP particles being retained in the topsoil, a combined result of particle and soil properties. Transport of larger MPs in clayey soils such as at our study site is highly dependent on the presence of large macropores. Consequently, we suspect that smaller MPs are more easily and preferentially transported downwards. While Py-GC/MS measurements enable rapid screenings for MP concentrations, micro-FTIR improves our understanding of the observed distribution and MP mobility. Combining these methods could potentially allow for more time-efficient, targeted MP monitoring in the future.

### 3.08.P-Tu203 Study of the Combined Effect of Microalgal Biomass and Microplastics in the Soil on the Retention of Three Pesticides

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Modern agriculture heavily relies on the addition of fertilisers on the fields and the use of pesticides to achieve the maximum crop yields per season. The most commonly utilised fertilisers are the chemically produced ones, while organic fertilisers such as manure as used to a lower degree. Another form of organic fertiliser has been proposed, e.i. microalgal biomass. The addition of microalgal biomass to the soil was found to promote plant and root growth and improve the fruit quality, and the elemental composition of the soil. From the sustainability point of view, the circularity of the nutrients is achieved if microalgal biomass from a nature-based wastewater treatment process is used. Microplastics (MPs) in agroecosystems mainly originate from soil amendments, mulching, surface runoff, and atmospheric deposition. MPs have been reported to play a role in the soil microbiome functioning, plant growth, and transport of chemical contaminants, like pesticides. This study aimed to determine the differences in the retention of three model pesticides in soil amended with microalgal biomass, and in MPs spiked soil amended with microalgal biomass. Selected model pesticides were acetamiprid (ACE; log Kow 0.80), chlorantraniliprole (CAP; log Kow 2.76), and flubendiamide (FLU; log Kow 4.20), while MPs particles used were polyethylene terephthalate, polystyrene, and polyethylene, in size range from 100  $\mu\text{m}$  to 1 mm. Pesticides solutions (1 mg/L and 5 mg/L) were added to the mixtures of soil-MPs, soil-microalga biomass, and soil-microalgal biomass-MPs to illustrate the pesticides' application rate of 2 mg/kg and 10 mg/kg, respectively. Solutions were left to stir, and after an hour, the concentrations of pesticides were determined by high-pressure liquid chromatography. Results showed that the overall retention of pesticides depended on the Kow of model pesticides (FLU > CAP > ACE) at both pesticide's application rates. At the higher application rate, we observed that the soil amended with microalgal biomass and polluted with MPs retained more pesticides compared to the soil only amended with microalgal biomass and also compared to the soil only spiked with MPs.

### 3.08.P-Tu204 Microplastics in Soil Systems, From Source to Path to Protection Goals

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Microplastics can be found everywhere in the world, and in any environmental compartment. During recent years it was discovered that most microplastics may end up in the soil compartment. While the amount of research on microplastics in the soil compartment has increased almost exponentially last 5 years, the number of scientific publications are lagging behind these on microplastics in the water compartment. There are indications that microplastics pose a risk to human health and soil life. Therefore, to inform policymakers and risk assessors, RIVM has summarized current knowledge and identified knowledge gaps of microplastics in the soil system. This poster presents the results of this inventory using a conceptual model which addresses topics considered most important for risk assessment. While the model includes several topics, the information presented here focusses on the topics sources, release and emission, fate and transport, and exposure.

### 3.08.P-Tu205 Iron Oxide Nanoparticles Enhance Plastic Biodegradation

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Bio-based plastics (BPs), produced from renewable biomass sources, are increasingly being used in commercial products and applications, especially bridgeable plastics. Therefore, their biodegradability and environmental fate continues to be an important subject of investigation. The soil bacterium *Ideonella sakaiensis* is owing a PETase enzyme capable to degrade conventional PET plastics. Fe<sub>3</sub>O<sub>4</sub> nanoparticles are regarded as safe material providing an essential element for bacterial growth. In this study, we explored beneficial properties of both *I. sakaiensis* and Fe<sub>3</sub>O<sub>4</sub> to degrade BPs PET and commercially available PET indicated by scanning electron microscopy (SEM), bacterial growth (CFU) and gene expression for PETase. Bacteria-plastic interaction was observed under epifluorescence microscopy and SEM characterized plastic surface. The first results show that after five days, bacteria degraded BPs PET to micro- and mm size debris. We presume that Fe<sub>3</sub>O<sub>4</sub> nanoparticles can enhance the BPs PET biodegradation. Further, we will perform the same experiment on commercially available PET to obtain a complete picture.

### 3.08.P-Tu206 Polyethylene Microplastics Influence the Distribution of Ivermectin in Soil

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Microplastics are found in almost every natural environment. A large number of studies has already investigated the role of microplastics as vectors for organic pollutants in aquatic ecosystems. However, the impact of microplastics on the distribution and transport of organic pollutants in soil remains unclear. In this study, we therefore investigated sorption and desorption of the hydrophobic antiparasitic agent ivermectin in soil under the influence of microplastics. Ivermectin is applied extensively in veterinary medicine worldwide and enters the soil via animal excretions. Batch sorption experiments were performed to evaluate the effect of low-density polyethylene (LDPE) microplastics (particle size 100 200 µm) at addition rates of 0% (control), 0.3% and 3% (w/w) in a reference soil (LUFA 2.4). The lower rate (0.3%) was selected as an environmentally relevant concentration of microplastics, while the higher rate (3%) represented an extreme scenario in the soil. The ivermectin concentrations assessed ranged from 15 to 1.500 µg L<sup>-1</sup> and covered two orders of magnitude. Samples were analyzed via high performance liquid chromatography using fluorescence detection (HPLC-FLD) after derivatization. Resulting sorption and desorption isotherms were fitted to the Henry model. The distribution coefficient for LDPE microplastics (K<sub>MP</sub>) in the soil-water system was calculated based on the highest amount microplastics added (3% LDPE). The addition of microplastics significantly influenced sorption of ivermectin in soil. Hence, distribution coefficients K<sub>D</sub> of 420 L kg<sup>-1</sup> (0% LDPE), 431 L kg<sup>-1</sup> (0.3% LDPE) and 586 L kg<sup>-1</sup> (3% LDPE) were calculated showing the highest sorption at 3% LDPE. Desorption of ivermectin in soil was similarly affected resulting in desorption coefficients K<sub>D</sub><sup>des</sup> of 643 L kg<sup>-1</sup> (0% LDPE), 719 L kg<sup>-1</sup> (0.3% LDPE) and 742 L kg<sup>-1</sup> (3% LDPE) with the lowest desorption at 3% LDPE. The K<sub>MP</sub> was 5.549 L kg<sup>-1</sup> indicating a high affinity of ivermectin for LDPE microplastics. The results imply that the presence of microplastics reduces the mobility of ivermectin in soil by increasing the sorption capacity and decreasing the desorption rate. LDPE therefore can be a sink for ivermectin in the soil environment. This may affect the uptake of ivermectin by plants and soil organisms such as earthworms.

### 3.08.P-Tu207 Plastic in Agricultural Production: Impacts Life cycles and Long-term Sustainability: Introducing PAPILLONS

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Plastics is an increasingly important commodity in agricultural production. However, some use and inappropriate waste management can result in the release of plastic debris to soils. The long-term impacts of these materials on soil ecosystem quality and functioning, and agricultural sustainability is uncharted. Knowledge and understanding of mechanisms and processes controlling releases, behaviour, fate and impacts of plastic pollution from both conventional and degradable polymers, as well as



their chemical additives on soil ecosystem and its productivity is mostly missing. PAPILLONS is a research and innovation action funded by the EU under the Horizon 2020 framework programme. The goal is to elucidate sustainability of plastic use in European agriculture in relation to releases and impacts of micro- and nano-plastics (MNPs) and their chemical additives in soil. Through novel applications of state-of-the-art analytical chemistry, materials science, and a series of multipronged experiments (from in-vitro to the field scale), we will elucidate sources, behaviour and impacts of particles in both micro- and nano-scales on agricultural soil ecosystems. We will take advantage of the cross-disciplinary synergies in integrative case studies, jointly addressing these knowledge gaps in different biogeographic, agricultural, and socioeconomic regions of Europe. Research tasks include: 1) inventorying usage of agricultural plastics and waste generation in Europe. 2) quantification of MNP release rate from prevalent agricultural plastics through ageing and fragmentation experiments; 3) assessments of MNP transport within soil and to groundwater, freshwater, biota and crops; 4) surveying current levels of plastic contamination in European agricultural soils; 5) elucidation of long-term impacts on soil properties and ecosystem services, looking at effects on biodiversity and functioning in soil invertebrate and microbial communities; 6) Assessment of stakeholder perspectives on the socioeconomic sustainability of plasticulture.

We address long-term implications of plastic pollution to tackle impacts on soil productivity, crop quality, agricultural yield. PAPILLONS uses a multi-actor approach whereby scientists in the areas of agronomy, environmental science, ecology, chemistry, engineering, economics and social sciences meet a diverse group of actors in the farming, industry, services and policy sectors to co-create knowledge.

### **3.08.P-Tu208 Shape as a Controlling Factor in Weathering of PET as a Precursor to Microplastic Formation**

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Plastic debris is exposed to a range of factors in the environment that lead to weathering of the material. The process of weathering results in changes in the material including surface cracking and enhances brittleness. Furthermore, weathering is considered a precursor to fragmentation and microplastic formation. However, the knowledge on the chain of these events is limited, especially how the form of the polymer affects the weathering. Therefore, a comparative study of the weathering process of polyethylene terephthalate (PET) was performed. PET in three different forms was exposed to UV light for three months. The samples included PET fibers, films, and pellets. Moreover, the effect of hydrolysis was studied by submerging half of the samples in water. The progress of weathering was investigated with scanning electron microscopy every 15 days. Additional analysis was performed on selected samples by Raman spectroscopy, x-ray diffraction analysis, and gel permeation chromatography. It was concluded that the weathering conditions as well as the specific properties of the plastics influence the development of the features related to weathering. It was shown that the combination of photo-oxidation and hydrolysis quickened the process. Sole hydrolysis did not seem to result in any damages on the material, while the exposure to the UV light led in changes in crystallinity and surface alterations, which were spotted in the final days of the experiment. While crack development was observed on pellets, films were indented with pits and fibers with grooves. The samples exposed to UV light while submerged in water started displaying signs of damage after 15 or 30 days of the weathering. While pellets immediately started to crack, films and fibers were first covered with pits, which then transformed into cracks. Each PET form showed a unique crack pattern, likely related to the production process. Moreover, the crack pattern was recognized to be a template to the surface layer ablation which was observed when additional mechanical stress was applied. Therefore, the size and quantity of the fragments could be estimated. It was suggested that 1.4-7.9 million microplastic fragments can be released from one cm<sup>2</sup> of a weathered PET film and 0.4-2.2 million from the same surface area of a pellet. Moreover, additional particles were seen emerging on the surfaces of the weathered samples that could be an additional source of microplastics produced during weathering.

### **3.08.PC Current State-Of-the Art in Understanding the Occurrence and Implications of Plastics in Terrestrial Environments**

#### **3.08.P-Tu170 Assessing the Impact of Microplastic Extraction Methods on Biodegradable Polymers**

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Agricultural soil is a sink for microplastics (MPs), with both indirect and direct sources. Indirect sources include the use of sewage sludge as fertiliser, plastic mulching films, atmospheric deposition, irrigation with wastewater etc. It is difficult to quantify the MPs addition from these sources, but the use of sewage sludge as fertiliser is estimated to add 63-430kt/year of MPs into European farmland (Nizzetto et al. 2016).

In comparison, the direct addition of MPs to soil can be quantified. In the EU, direct sources (e.g., the application of controlled release fertilisers, and plant protection products) account for approx. 23,500 tonnes/year of MPs, of which plant protection products account for approx. 1,000 tonnes/year (ECHA, 2019). This is preventable and under new restrictions proposed by the European Chemicals Agency, biodegradable polymers are suggested as future alternatives (ECHA, 2019). It is likely that biodegradable polymers will be used in soil applications and therefore essential that robust extraction and analytical methodologies are developed to monitor their environmental fate. However, such methods for the analysis of MPs in soil have not generally been designed for biodegradable polymers. For environmental soil samples, a combination of density separation with ZnCl<sub>2</sub> and enzymatic-oxidative digestion is a promising technique that has been shown to have a high removal efficiency for soil organic material (e.g., Möller et al. 2020).

In this work, enzymatic digestion has been tested on representative polymers polylactic acid (PLA) and poly(r)-3-hydroxybutyrate (PHB). Polymer films were made from powder, then their stability after exposure to the enzymatic-oxidative digestion method was determined. Impacts of each treatment were assessed visually via microscopy and quantitatively by weight and thickness measurements. Surface degradation was measured with attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), and polymer molecular weight was measured by gel permeation chromatography (GPC). Results show variability in the impact on the polymer depending on the treatment used and the application sequence. A combination of both visual assessment and chemical characterisation was needed to determine impacts on polymers. This work highlights the importance of quantifying the impacts of microplastic extraction methods on specific polymers of interest to improve future method development.

### 3.08.P-Tu171 Suitability of Elutriation for the Extraction of Microplastics from Natural Soils

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We evaluated the suitability of elutriation for improving the extraction of microplastics as a function of soil textures. Microplastic pollution in marine environments has been extensively studied in modern literature; however, information about the prevalence of microplastics in freshwater and terrestrial systems is lacking. The lack of published literature regarding the extent of microplastic contamination of terrestrial ecosystems is in part due to the lack of any standardized extraction methodology for microplastics from high-clay and organic-rich soils. In this study, we obtained five samples with textures varying from, 0.1-8.6% organic matter, 16.7-96.3% sand, 2.5-45% silt, and 1.3-50.0% clay and compared the extraction efficiency of an extraction process that included elutriation with a typical direct digestion process. All soils were subjected to microplastic extraction with and without elutriation (direct digestion). Samples were digested in 7.5% NaOCl, then flotation in 5.1M ZnCl<sub>2</sub> solution. The number of particles was quantified through staining and fluorescence microscopy. The amount extracted varied from 3,300-188,900 particles/kg in elutriated soils and from 3,600-153,700 in direct digested soils. Linear regression was used to determine the relationship between relative number of particles extracted (with elutriation vs without elutriation) and combinations of soil parameters. Correlations were observed between relative extraction quantity and clay content ( $R^2=0.64$ ), (clay+OM)/(silt+sand) ( $R^2=0.69$ ), (clay+OM)/silt/sand ( $R^2=0.81$ ), (clay+OM)<sup>1/2</sup>/silt/sand ( $R^2=0.88$ ), suggesting that elutriation may be effective as an extraction step for high-sand and silt soils, and should be avoided for high-clay and organic matter soils. Currently, we are validating the plastic composition of the particles using Attenuated Total Reflectance – Fourier Transformed Infrared spectroscopy and quantifying the amount of remaining metals and salts using Inductive Coupled Plasma Mass Spectroscopy and Emission Dispersive X-ray spectroscopy. The information gathered in this study should help other groups in determining the extent of microplastic contamination in Earth's ecosystems by providing viable extraction methodologies for a range of soil types. A rigorous understanding of microplastic prevalence in the environment will aid in determining the impacts associated with microplastic contamination.

### 3.08.P-Tu175 From Wastewater Treatment Plants to the Terrestrial Environment: Microplastics in Sewage Sludge Compost, Agricultural Soil, and Earthworms

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Wastewater treatment plants have been found to be a significant source of microplastics to the terrestrial environment. After wastewater purification, most of the microplastics in the wastewater influent end up into the resulting sewage sludge, which then can be processed further, for example, into compost. The microplastics enter the environment when the compost is applied as a fertilizer to a soil. From the soil, the microplastics can be digested by soil dwelling organisms, like earthworms. The objectives of this study were 1) to develop sample preparation methods for isolating microplastics from sewage sludge compost, soil and earthworm samples, and 2) to investigate the number concentration of microplastics in these samples. Sewage sludge compost, agricultural soil fertilised with sewage sludge compost, control agricultural soil without compost fertilizer and earthworms taken from the same soils were sampled for microplastics. Sample preparation was tailored to each of these specific sample matrices with methods including sieving, filtering (20 µm), organic matter removal and density separation. The prepared sample was then filtered onto an Anodisc filter (pore size 0.2 µm, diameter 25 mm). An imaging Fourier Transformation Infrared microscope (µFTIR; Lumos II, Bruker) was used to identify the microplastics in transmittance mode. Particular effort was put to mitigate sample contamination. Sample preparation and FTIR-analysis were conducted in a separate, synthetic-fibre-free laboratory meant to be used only for microplastic research. Laboratory blank samples were handled similarly with the real samples to assess the sample contamination from the laboratory environment. In this presentation, the number concentration of microplastics in sewage sludge compost, agricultural soils and earthworms are shown and the links between the microplastic sources and sinks are discussed.

### 3.08.P-Tu189 Effects of Conventional and Biodegradable Microplastic on Seed Germination and Plant Growth of Food Crops

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Nano- and microplastic (<100 nm and <5 mm, respectively) pollution is becoming increasingly persistent in all ecosystems and is considered an emerging threat to biodiversity and ecosystem functioning. One major concern are agricultural lands, which have been identified as long-term microplastic sinks. As a result, there is now a push towards developing bioplastics, which can be

(partly) broken down after entering the environment. Yet, limited research has been done to compare the impacts of microplastics and bioplastics on terrestrial crop plants. This study aims to examine and compare the effects of artificially weathered conventional polyethylene (PE) and starch-based biodegradable polybutylene adipate terephthalate (PBAT) on four common food crops, including lettuce (*Lactuca sativa* L.), carrot (*Daucus carota*), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare*), through two complimentary experiments. We investigated the effects of environmentally relevant low, medium, and high (0.01%, 0.1%, 1% w/w) concentrations of weathered PE and PBAT particles on seed germination (acute toxicity: Petri-dish experiment) and plant growth (chronic toxicity: pot-plant experiment) respectively. Our results show adverse effects of both PE and PBAT on crops. When acutely exposed during germination, PE significantly accelerated the germination rate of carrot seeds by two days ( $p < 0.05$ ). In addition, both PE and PBAT decreased the root length of lettuce and wheat by up to 36% and 53% respectively ( $p < 0.05$ ). Contrary, root length ( $p < 0.0001$ ), shoot length ( $p < 0.05$ ) and seedling biomass ( $p < 0.05$ ) of carrot were increased with up to 86% when exposed to PE and PBAT respectively. In the pot-plant experiment, PBAT significantly delayed the germination rate of barley ( $p < 0.05$ ), and additionally decreased the shoot biomass by 16% after 14 days ( $p < 0.05$ ). No significant effects of PE and PBAT were recorded on the seed germination of lettuce. Overall, these results show that the early development of seedlings are mainly impacted, but over time plants might recover mildly. Moreover, biodegradable plastic was more toxic than the conventional plastic highlighting the need to further investigate the effects of micro- and bioplastics on crop plants.

### 3.08.P-Tu190 Long-term Effects of Agricultural Microplastics on Earthworm *Eisenia Andrei*; Comparison of Conventional and Biodegradable Plastics

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Use of plastics is common in modern agriculture, but degradation of plastics in soil is a slow process. Biodegradable plastics have been proposed as a solution for this problem, but little is known about possible long-term effects of plastics, either biodegradable or conventional, on soil organisms. The aim of our study is to find out the possible effects of microplastics from conventional and biodegradable mulching film material on the earthworm *Eisenia andrei*.

We studied two types of microplastics produced by grinding pellets made of recycled mulching films, one from conventional polyethylene (PE) films and the other from biodegradable films composed of starch and polybutylene adipate co-terephthalate (starch-PBAT). We exposed earthworms to these two microplastic types in concentrations of 0 %, 0.005 %, 0.05 %, 0.1%, 0.5 %, 1 % and 5 % of dry mass. The experiments started with standard reproduction tests with earthworms of parental (P) generation (ISO 11268-2). After the standard tests, we selected 13 juveniles from each test jar for new set of tests to study the impacts of long-term microplastic exposure on the first offspring generation (F1). We followed the growth and the number of mature individuals every four weeks. In addition to survival, growth and reproduction, the uptake of microplastics and plastic additives as well as immunological biomarkers in earthworms will be analyzed.

The experiments are still running, and statistical analyses have not been performed yet. The preliminary data suggest that the microplastics have not affected earthworm survival, but the presence of PE in the soil decreased the growth in F1 generation. In contrast, the biodegradable plastic seems to increase the growth of the earthworms in F1 generation. The earthworms in the highest PE concentrations also matured later.

This study is part of a PhD project which focuses on the long-term effects of microplastics on earthworms in agricultural soils.

### 3.08.P-Tu202 Microplastic Distribution in Sewage Sludge Amended Soil - The Advantage of Complementary Uses of Py-GC/MS and micro-FTIR Imaging

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Sewage sludge application to agricultural land has been associated with elevated microplastic (MP) loads on soil. Although laboratory studies suggest that microplastics may be transported to deeper soil layers by bioturbation and percolation in macropores, these transport processes have hardly been assessed in field trials yet. There is furthermore a strong divide between studies, either applying spectroscopic methods focussing on particle counts and properties, and thermoanalytical techniques which primarily yield total polymer masses. Field studies analysing both the mass distribution of MPs in soil and particle shapes and sizes are still scarce but needed to better understand their distribution in soil.

We analysed soil core samples along 90 cm depth profiles from agricultural long-term field studies in Sweden to which sewage sludge was applied since 1996. First, we used solvent-based pyrolysis gas chromatography – mass spectrometry (Py-GC/MS) to identify samples of interest. Samples with exceptionally high MP concentrations were subsequently analysed with micro-Fourier Transform Infrared spectroscopy (micro-FTIR) imaging for characterizing sizes, polymer type, and shape associated parameters. The highest MP mass concentrations were found in and directly below the plough layer, successively decreasing with depth, but still detectable at 40 cm depth. We inferred that the observed decreasing mass concentration with depth is probably a result of larger MP particles being retained in the topsoil, a combined result of particle and soil properties. Transport of larger MPs in clayey soils such as at our study site is highly dependent on the presence of large macropores. Consequently, we suspect that smaller MPs are more easily and preferentially transported downwards. While Py-GC/MS measurements enable rapid screenings for MP concentrations, micro-FTIR improves our understanding of the observed distribution and MP mobility. Combining these methods could potentially allow for more time-efficient, targeted MP monitoring in the future.

### 3.08.V Current State-Of-the Art in Understanding the Occurrence and Implications of Plastics in Terrestrial Environments

### 3.08.V-01 Can Wastewater Treatment Plants' Mitigate Microplastic in Terrestrial Environment? – A Comparative Study of Sludge Treatments

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The aim of this study is to quantify the efficiency of different sludge treatments capabilities of removing microplastic (MP) from sewage sludge at wastewater treatment plants (WWTPs).

Sludge treatments are the last stop before sludge fraction leaves the WWTP. ~99% of MP entering WWTPs is presumed to be retained in the sludge fraction, hereby becoming a relevant potential route for environmental exposure, if MP is not removed in the sludge treatment. Sludge is worldwide used as fertilizer on agricultural fields, and MP in sludge fraction can both influence the terrestrial environment as well as being transported to the aquatic environment.

At WWTPs sludge treatments primary function is to aggregate solid fraction in sludge and reduce water content. However some sludge treatments also have the ability to influence the concentrations of MP in sludge, hereby reducing the environmental impact. New technologies such as Pyrolysis are emerging as a new way of removing contaminants and could potentially remove MP at WWTPs.

Standard practice today includes mechanical dewatering, where polymer emulsion can be added to the process to increase the dewatering process. This could result in an increased MP abundance.

This study aims at assessing the effectiveness of different sludge treatments in removing MPs in sludge. This is done by assessing MPs in sludge pre and post treatment with biological degradation technologies (Sludge mineralization/Reedbed systems) and gas producing technologies (Incineration, pyrolysis and anaerobic digestion) as well as mechanical dewatering.

This also includes an analysis of polymer emulsion used at the WWTPs to determine whether these products increase MP abundance in sludge fraction.

Samples of sludge before and after treatment are gathered from five different WWTPs, purified and analysed using FTIR to determine plastic polymers and concentrations, generating the much-needed understanding of how these treatment technologies can help minimize the MP contamination emitted from WWTPs.

### 3.08.V-02 Towards Quality Assured Measurements of Microplastics in Soils Using Fluorescence Microscopy

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Monitoring microplastics (MP) in agricultural soil has become an area of great research interest, given the potential hazards posed by microplastics to the soil ecosystem and human health. Measuring particle number concentration is essential in this context, particularly for ecotoxicology. Various analysis methods have been developed so far, albeit the results are often inconsistent, difficult to compare and uncertain. Among these methods, fluorescence spectroscopy-Nile Red staining has progressed as a low-cost, relatively simple-to-use approach for analysing a broad spectrum of microplastics in complex environmental matrices like soils. However, validated sample preparation and analytical methodologies, with quality controls and assurance, are often lacking and difficult to establish but are essential for reliable measurements. Therefore, this study aims to establish an assured, reproducible fluorescent-based methodology for analysing MP in soil, which discriminates MP size and type present in different soil types. Eight MP types (including both non-biodegradable and biodegradable plastics) within three size categories (dia. 500-1000 µm, 100-250 µm and 10-150 µm) were "spiked" into three different agricultural soils (loam-reference soil, clay, and sandy soils) for a comprehensive assessment of the fluorescence microscopy methodology. As a tool to shorten the analysis time, a digital image analysis pipeline using Image J was developed, which allowed for fully automatic quantification of MP in the samples. Validation of the ImageJ method shows a high accuracy (88% match to true observation) for MP particles on a filter without a soil matrix. Recoveries ranged from 80-90% for MP with sizes from 500-1000 µm regardless of the soil types, whereas those for smaller MP (10-250 µm) varied between different soils and plastic types, with the lowest values observed in clayey soil and for biodegradable plastics. The results probably reflect the complexity of clay soil owing to a high presence of soil organic matter and very small and negatively charged mineral particles and the possible degradation of biodegradable plastics during their extraction from the soil. This helps to better understand the MP loss during sample preparation and due to the nature of the soil and plastic interactions. A relationship between the microplastic mass and the corresponding particle number was established in this study, which enabled the conversion between mass and particle number data.

### 3.09 Emerging Organic Contaminants in the Oceans: Local Sources, Long-Range Environmental Transport and Impact of Climate Change

#### 3.09.T-01 Exposing Marine Microbial Communities to Organophosphate Ester Flame Retardants and Plasticizers Along the Atlantic Ocean

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The widespread usage of organophosphate esters (OPEs) as flame retardants and plasticizers has led to their ubiquitous presence in the environment. This fact, together with their potential toxic effects on humans and ecosystems, has caused them to be considered organic pollutants of emerging concern. In addition, OPEs are potential phosphorus (P) sources that can cause an anthropogenic perturbation of the P marine biogeochemical cycle. However, no international regulation exists to tackle the increasing emissions of OPEs. It is necessary to understand their biogeochemistry in order to improve risk assessments and regulation.

To help understand the biogeochemistry of OPEs in the open ocean we performed a series of experiments during the ANATOM oceanographic cruises with the objective of determining the role that marine bacteria play (usually underestimated) in the fate of these compounds. Briefly, these experiments consisted of incubating for 48h at dark 2L of surface seawater where a mixture of 6 known OPEs had been spiked. We measured the OPE concentrations at the beginning and at the end of the incubations and we characterized the bacterial populations by 16S Amplicon sequencing. Additionally, we measured prokaryotic cell abundance, bacterial production, and inorganic nutrient and total organic carbon (TOC) levels. Concurrently, bacterial production was used to estimate nutrient limitations in each experiment in short-term nutrient enrichment bioassays.

Here I present the first results of the experiments carried out during the ANATOM-1 oceanographic campaign, which consisted of a longitudinal transect across the Atlantic Ocean. Bacterial production results, estimated as incorporation rates of  $H^3$ -leucine, showed an increase in the bacterial activity after 48 hours of incubation. The analysis of the bacterial community composition resulted in an enrichment of at least 8-fold of the genera *Alteromonas*, *Pseudoalteromonas*, and *Sulfitobacter*. Although there is no evidence in the literature of these genera being able to degrade OPEs, all of them are known to be opportunistic taxonomic groups. Data from the quantification of the OPE levels and other measurements will allow us to understand what triggered these changes.

These experiments will shed light on the interactions between marine bacteria and OPEs in the open ocean under different biogeochemical regimes, and will help understand the biogeochemistry and fate of OPEs in the environment.

### 3.09.T-02 Investigating the Presence and Impact of Pharmaceuticals in Stony Corals from Shallow and Deep Sites in the Red Sea

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The increasing global use of pharmaceutically active compounds (PhACs) and their insufficient removal by sewage treatment plants results in their chronic input into coastal waters, where they pose a threat to marine organisms. Numerous studies investigate the presence and effects of PhACs in various marine organisms. However, available data regarding the presence of PhACs in corals worldwide is scarce. In a recent study, 14 antibiotics were detected in coastal corals from the South China Sea in concentrations of 28 ng/g dry weight tissue. Moreover, information regarding the effects of PhAC contamination on corals is highly limited, in comparison to assessment of the hazardous effects of heavy metals, microplastics and personal care products. The main goal of this study is to examine the presence of PhACs in corals from the Israeli Red Sea coral reefs and investigate their potential effects according to their mode of action. Eighty coral samples of the branching *Acropora* sp. and the massive *Favites* sp. were collected from four shallow and deep sites (5-12 m and 30-40 m, respectively). Chemical analysis is currently in progress and will include 25 compounds from the most common subscribed drugs in Israel, as reported by the main health services providers. Isotopic labeled standards will be used to quantify recovery rates and validation. Samples will undergo lyophilization, Accelerated Solvent Extraction (ASE), Solid Phase Extraction (SPE) and analysis will be performed by Ultra-Pressure Liquid Chromatography (UPLC) coupled with a Triple-Quad MRM Mass Spectrometer. Preliminary results demonstrate the presence of 14 out of 14 analyzed PhACs in Red Sea coral samples. In addition, in order to understand the potential physiological effects of PhACs, alteration in protein expression in response to PhACs exposure, under controlled laboratory conditions, will be examined in *Favites* sp. and *Acropora* sp. samples. Each coral species will be exposed to a mixture of environmentally detected PhACs for 14 days in different concentrations; 0=control, X (environmentally relevant concentration), 10X, and 100X. Total proteins will be extracted and processed at the Smoler Proteomics Center, Technion, Haifa. Protein profiles will be analyzed using Perseus software. The study will yield essential information regarding the presence of various PhACs in stony corals from a tropical ecosystem and the potential effects of PhACs contamination on coral physiology.

### 3.09.T-03 Short, Medium and Long-chain Chlorinated Paraffins and Dechloranes in Marine Mammals from Norway

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Marine mammals are considered sentinel species for the marine ecosystem health due to the bioaccumulation of legacy and emerging contaminants in their tissues. Data on the presence, levels and distribution of many chlorinated paraffins and dechloranes, however, remain missing or incomplete for many whale species from Norway. The aims of this study were 1) to conduct the first screening of chlorinated paraffins and dechloranes in marine mammals from Norway and 2) investigate inter-species differences due to trophic position. We collected samples from 32 stranded whales of nine different species from Norway 2015-2020, including one neonate killer whale (*Orcinus orca*) of approximately 10 days of age, in addition to 14 minke whales obtained in 2019 from the annual harvest in the Barents Sea.

Levels of short chain chlorinated paraffins ranged from 10 ng/g lw (minke whale) to 1575 ng/g lw (sperm whale), medium chain from 2 ng/g lw (fin whale) to 2383ng/g lw (sperm whale) and long chain chlorinated paraffins from 2 ng/g lw (harbour seal) to 1093 ng/g lw (minke whale). Levels were highest in the minke, killer and sperm whales, with approximately three fold higher levels in subadult female minke whales than male minke whales. All chlorinated paraffins were also detected in the neonate killer whale, in levels similar to adults, which gives the first evidence of maternal transfer in killer whales.

Dechlorane 602 was the only dechlorane found in all of the samples, and we found the highest levels in the killer whales (mean 4.8 ng/g lw, excluding one individual suspected of eating seals with a level of 79 ng/g lw), followed by the sperm whales (1.8 ng/g lw). The lowest levels were found in the humpback whales (mean = 0.05 ng/g lw). The killer whale neonate had approximately three times lower levels than adults.

We found higher levels of chlorinated paraffins and dechloranes than other Arctic marine mammals. Our study illustrates the importance of regular screenings of contaminants in Arctic wildlife.

### 3.09.T-04 Sand and Mussels as Indicators of Volatile Methylsiloxanes in Coastal Areas

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Volatile methylsiloxanes (VMSs) have been recently identified as emerging organic contaminants with potential harmful effects to the environment. Due to their high vapor pressure and compatibility with numerous ingredients used in formulations, they are widely used in numerous domestic and industrial applications, highlighting its intensive use in personal care products (PCPs). This is one of the main reasons for the presence of these compounds in the environment, namely in coastal areas. The increased use of these products particularly in summer, due to sunscreens and other cosmetics, has led to the need to study the associated anthropogenic impact and a possible time trend.

In this work, sand and mussels were chosen to give an idea of the presence of seven VMSs (3 linear: L3,L4,L5 and 4 cyclic: D3,D4,D5,D6) in mineral and biota matrices. The analysis in both cases was done by optimizing and validating a QuEChERS extraction protocol, with the quantification done by GC-MS.

Individual VMSs were detected in all sand samples with concentrations ranging from n.d. to 33±7 ng/g dw. As expected, cyclic VMSs were detected with greater frequency and concentration than linear, namely the less volatile ones, such as D5 and D6, which is probably associated with the higher frequency of deposition and permanence in the sand. In general, VMS levels were higher in the Canary Islands (Spain) than in other countries. VMSs were detected also in all mussel samples, and the cyclic ones in higher levels, again D5 and D6 (max 53±12 and 27.2±0.8 ng/g ww, respectively).

The geographical distribution of VMSs in coastal areas is linked to the temporal trends, as the warmer months tend to attract much more people to these areas than in the rest of the year. Moreover, intra-day study showed that VMS levels tend to follow the beach attendance. Mussels follow a similar trend, but with the important finding that the depuration made before commercialization is key to reduce the exposure of humans to VMSs by ingestion.

### 3.09.T-05 Long-range Environmental Transport of Legacy and Emerging Per- And Polyfluoroalkyl Substances in the Global Ocean and Polar Regions

**Zhiyong Xie, Helmholtz Center Hereon, Germany**

Owing to the unique properties, per- and polyfluoroalkyl substances (PFASs) can be transported from continental sources to remote areas through both atmosphere and ocean currents, and have been widely presenting in all environmental compartments in remote Arctic and Antarctic. The neutral PFAS, such as FTOHs, Me/EtFOSE and FOSE mainly partition to air, and travel with air masses from source regions to the remote oceans. While ionic PFASs mostly accumulate in surface waters, and their long-range environmental transport. In the polar regions, photodegradation and reaction with OH, Cl or O<sub>3</sub> radicals are the major pathways to eliminate neutral PFAS from the Atmosphere and transform to ionic PFAS, especially in spring and summer. Moreover, reemission of PFAS from cryosphere may lead high levels in the coastal waters of the arctic and Antarctic. The objective of this work is to present the spatial distribution and temporal trends of legacy PFAS and to highlight the occurrences of the emerging PFASs in the Atlantic ocean and polar regions.

### 3.09.P Emerging Organic Contaminants in the Oceans: Local Sources, Long-Range Environmental Transport and Impact of Climate Change

#### 3.09.P-Mo208 Where to Look – Stability and Seawater-particulate Partitioning of PPCPs

**Lisbet Sorensen<sup>1</sup>, Ingrid Alver Hovsbakken<sup>2</sup>, Mari Creese<sup>1</sup>, Ewa Wielogórska<sup>1</sup>, Antonio Sarno<sup>1</sup> and Ida Beathe Overjordet<sup>1</sup>,** (1) SINTEF, Norway, (2) Norwegian University of Science & Technology (NTNU), Norway

Pharmaceuticals and personal care products (PPCPs) the environment via wastewater from urban, domestic, and industrial areas, in addition to sewage, aquaculture and agriculture runoff. Many PPCPs are only partly removed in water treatment, meaning small amounts are continuously released into the environment, and environmental concentrations worldwide are reported in the ng-µg/L range.

While some PPCPs are easily degradable in environmental conditions, others are more persistent, meaning they are less easily degraded and can stay in the environment for long periods of time. Degradation is dependent on parameters such as physical and chemical properties of the individual compounds, and environmental conditions. Many pharmaceuticals are already known to be able to adsorb to sediment particles and these mechanisms can act as a sink for accumulation of these compounds. If pharmaceuticals adsorb and migrate into marine sediments, their persistence can increase in comparison to their persistence in the water column. This can affect their bioavailability and transport pathways.

By exploring the stability and adsorptive properties of PPCPs onto particles relevant for marine conditions, we can better understand their environmental behaviour and transport potential. This study investigated the PPCP sorption capacity of inorganic (kaolin) and biotic (*Rhodomonas baltica*) marine particles, and further investigated the adsorption mechanisms between pharmaceuticals and kaolin, through exploring adsorption kinetics and isotherms.

Overall it was demonstrated that many PPCPs are stable in seawater for long enough to be transported and interact with marine particles. None of the PPCPs sorbed to the algae in the batch experiment, and thus further investigations focused on determining

sorption kinetics and isotherms to the inorganic particle kaolin. Sorption kinetics revealed a better fit of the pseudo-second order model than the pseudo-first order. The sorption rates increased with increasing amounts of particles in the system. The isotherm experiments revealed that the experimental data fit best to the Sips isotherm, a 3-parameter model. The 2-parameter Langmuir model also fit the data well. The Sips model revealed a temperature dependence for the Sips affinity constant,  $K_S$ , and the maximum adsorption capacity,  $q_{\max}$ .  $K_S$  decreased with increasing temperatures, while  $q_{\max}$  increased.

### **3.09.P-Mo209 Addressing the Importance of Microplastic Particles as Vectors for Long-range Transport of Chemical Contaminants: Perspective in Relation to Prioritizing Research and Regulatory Actions**

**Todd Gouin**, *TG Environmental Research, United Kingdom*

Over the last several years there has been increasing concern regarding the environmental fate and potential global transport of plastic debris, particularly in the form of microplastic particles (MPs). The global transport of MPs has also triggered concerns regarding the potential role that its mobility may represent towards influencing the long-range environmental transport (LRET) of particle-bound chemicals, particularly the large number of chemicals known to be added to plastic. This presentation considers the various lines-of-evidence that might be used to demonstrate the LRET of MPs, such as data reporting their presence in remote locations and models that have been developed to estimate their environmental fate and transport. When considering the available information, several challenges and uncertainties can be identified that limit the ability to differentiate local versus global sources of plastic debris and MPs in the environment and their potential to act as vectors of LRET for plastic additive chemicals (PACs). There is thus an urgent need towards a coordinated strategy for the development of holistic mechanistic modeling and standard sampling and analytical methods that can strengthen our overall understanding of the environmental fate, transport, and exposure of MPs. Knowledge of the polymer composition, size and shape of MPs and their potential mobility under different environmental conditions can be used to a) identify potential geographic hotspots and b) evaluate the types of PACs that may be associated with those materials and which can be used to help inform the application of models for characterizing and quantifying their leachability. When combined with derivation of emission factors of PACs from in-use products and subsequent environmental release scenarios, a holistic exposure assessment may potentially be realized that strengthens and improves our overall understanding of environmental human health implications related to the environmental fate of MPs.

### **3.09.P-Mo210 Understanding the Source and Environmental Fate of Tyre Wear Emissions**

**Nick Molden and Ime Usen**, *Emissions Analytics, United Kingdom*

Tyres are complex products, with sophisticated physical design and chemical composition. They must deliver a range of often conflicting performances including slow wear (for maximum life), low rolling resistance (to reduce carbon dioxide emissions and fuel consumption), wet grip and low noise. There exist regulatory and labelling requirements around these. Where information is currently lacking is in the wear rates and chemical composition of the tyres. This is relevant to the amount material released into the environment, where it goes and what damage it does to human and animal health, and the environment more widely. The demonstrated link between the 6PPD preservative in tyres and the death of salmon and trout in the US is important context to this work.

The context of this work is emerging regulation of tyre wear, from the proposed banning of 6PPD in California to the potential UNECE/EU regulation of tyre wear rates. Research is currently limited on the nature of tyre wear particles and in what proportions they get distribution into air, water and soil.

This presentation will focus on understanding the nature of tyre wear emissions, by first of all understanding the chemical composition of original tyres. It will draw on Emissions Analytics' tyre material database, which typically identifies over 400 organic compounds in each tyre, using its optimised process of thermal desorption and pyrolysis, coupled with two-dimensional gas chromatography and time-of-flight mass spectrometry. It will go on to perform equivalent chemical analysis on tyre wear material collected from vehicles in real-world operation, and relating that back to the composition of the original tyre.

Finally, an approach to assessing the presence of tyre wear particles collected in environmental matrices will be shown. It will move beyond using a small number of tracer compounds to determine environmental distribution, towards a fuller multi-compound 'fingerprinting' approach using the ability of two-dimensional chromatography.

### **3.09.P-Mo211 Quantitative Screening of Organic Plastic Additives in Beach Sand Using QuEChERS**

**Javier Castro-Jimenez<sup>1</sup>** and **Nuno Ratola<sup>2</sup>**, *(1)Chemical Contamination of Marine Ecosystems, IFREMER, France, (2)Universidade do Porto, Faculdade de Engenharia, Portugal*

This work presents an expedite and environment-friendly analytical protocol based on QuEChERS technology for the simultaneous determination of organophosphate and phthalate esters (OPEs and PAEs, respectively) plastic additives in beach sand, a pollution indicator of coastal areas, often under important anthropogenic pressure. OPEs and PAEs are among the most widely distributed organic plastic additives in the marine environment, which may present a risk for the wildlife and human health. The QuEChERS protocol used is an adaptation of a previously reported method allowing the quantitative screening of OPEs and PAEs in other marine matrices, such as sediments and marine biota, optimized now for the analysis of these contaminants of emerging concern in beach sand. The application of the protocol to real samples included validation via recovery and repeatability assays. A total of sixteen additives (9 OPEs and 7 PAEs) were analyzed by GC-MS. Method recoveries at two spiking levels (25 and 100 ng sample<sup>-1</sup>) varied from 76 ± 30% to 58 ± 15% (mean ± SD) for OPEs and from 64 ± 16% to 60 ± 10% for PAEs. Mean (n=3) relative standard deviations ranged from 4.4 ± 0.9 % to 10.6 ± 0.8 %, indicating a good method repeatability. Results from an initial screening in selected beach sand samples collected in NW Mediterranean Sea (Marseille, France) confirmed the presence of both OPEs and PAEs in this matrix. Overall, most PAEs were detected (detection frequency, DF=86%) and concentrations ranged from 21 to 29 ng g<sup>-1</sup> dw (sumPAEs). OPEs exhibited a lower DF (33-67%) and

concentrations were also lower compared to PAEs, varying from 2 to 4 ng g<sup>-1</sup> dw (sumOPEs). Tris-(2-chloro, 1-methylethyl) phosphate (TCPP) and diethylhexyl phthalate (DEHP) exhibited the highest concentrations among OPEs and PAEs, respectively. These two additives have been reported as hazardous chemicals and are also classified (or suspected) endocrine disruptors. Despite the limited number of explored beaches, these results show the applicability of our method to investigate the environmental occurrence of these plastic additives in sand. A wider survey, considering also beaches from different European countries is ongoing.

### 3.09.P-Mo212 Watch List of Substances under the Water Framework Directive: Work in Progress

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The watch list of substance is articulated through implementing decisions determined by the Commission and it is updated every two years. The first list was published in 2015 (EU Decision 2015/495) and contained ten substances or group of substances. In the subsequent updates (EU Decision 2018/840, EU Decision 2020/1161 and EU Decision 2022/1307), some of the substances were removed from the list, and some other were identified as suitable candidates to be included in it. Many of these substances, including herbicides, pesticides, fungicides, personal care products, pharmaceuticals such as antibiotics, antidepressants, and fungicides among others, are substances in everyday use, but their effects on the environment are little or no studied at all. The continuous monitoring period for each substance included in the watch list should not exceed four years, and the monitoring of substances must obtain high quality data on their concentrations in the aquatic environment, which implies having validated and consolidated methods.

These constant changes do not allow a good consolidation of analytical methods, so that when a method have been optimised, it has to be modified to include the determination of the new substances, or a new method have to be developed. In addition, if methodology changes, consistent time series beyond 4 years are difficult to obtain.

In the Basque coast, southwest Bay of Biscay, the Basque Water Agency (URA) has been promoting the study of emerging substances on the watch list since 2017. Grab water samples have been collected quarterly (spring, summer, autumn, winter) at emission points of some wastewater treatment plants, and at their receiving waters (transitional or coastal waters).

Obtained results, available upon request to URA, could be useful for the purpose of supporting the determination of the risk these substances could pose to the environment, and the prioritisation of substances to be included in the future list of priority substances.

### 3.09.P-Mo213 Spatial Distribution and Time Trends in Cyclic and Linear Siloxanes in Sediment From Semi-enclosed Bays of Korea Between 2013 and 2021

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Siloxanes are contaminants of emerging concerns in aquatic environment. Sediment was collected from semi-enclosed bays of Korea in 2013 and 2021 to grasp the spatial distribution, potential sources, and time trends in siloxanes. Cyclic and linear siloxanes were detected in all sediment samples, indicating ubiquitously contaminated. Highest siloxane concentrations were observed in sediment near the outfall of wastewater treatment plant, implying a major source of siloxanes to the coastal environment. The concentrations of cyclic and linear siloxanes in sediment were not changed between both years, indicating continuous contamination for the last two decades. The consumption of siloxanes in the Korean industrial market is still large and continues to increase. Multivariate statistical analysis showed the different contamination patterns of siloxanes, depending on the sampling locations and industrial types. All sediment samples were dominated by cyclic siloxanes, such as D5 and D6 because the Korean industrial market has been dominated by cyclic siloxanes.

### 3.09.P-Mo214 Per- and Polyfluoroalkyl Substances (PFAS) in Flatfish and their Prey: Occurrence and Trophic Transfer

**Yann Aminot<sup>1</sup>**, Nadège Bely<sup>1</sup>, Guillaume Bernard<sup>1,2</sup> and Aourel Mauffret<sup>1</sup>, (1)IFREMER, France, (2)University of Bordeaux, France

Submarine continuations of river deltas, such as marine prodeltas and mud patches, are key components of the land-sea continuum which can be geographically separated from river estuaries. Such highly dynamic systems are submitted to both natural and anthropogenic disturbances, and are essential areas for juvenile stages in the life cycle of most marine exploited species, but remain poorly studied with regards to contaminant transfers. We study here the West Gironde Mud Patch (SW France), taken as a model of marine offshore system under anthropogenic pressure. Among contaminants of emerging concern, per- and polyfluoroalkyl substances (PFAS) are a family of thousands of non-naturally occurring chemicals reported for their ubiquitous presence in the environment. Evidence for their persistence, bioaccumulative and toxic properties has led to major concerns, prompting a global call for a better management of this chemical class as a whole. PFAS stability in environmental waters and a rather limited affinity to particles has made oceans the largest environmental reservoir of historically released PFAS. Conversely, there is still a dramatic lack of knowledge on the exposure of marine biota to PFAS, and on their subsequent bioaccumulation and potential toxic effects, beyond the most studied PFOS and long-chain perfluorocarboxylates (PFCAs). In this presentation, we describe the occurrence and profiles of PFAS in the common sole *Solea solea* and in benthic invertebrates composing its diet. We conducted a thorough sampling of over 17 benthic species from the studied site in two contrasted seasons. Perfluorosulfonates, PFCAs and precursor compounds were analysed by liquid chromatography coupled to tandem mass spectrometry after a 2-step clean-up of the extracts. Our results indicate relatively high levels of PFAS in such offshore



environments, with average PFOS concentrations in sole muscles at  $3.4 \pm 1.9$  ng/g dw, and sum C<sub>8-15</sub> PFCA at  $4.3 \pm 4.1$  ng/g dw. The precursor compounds FOSA and n:3 FTCAs were also observed. In benthic invertebrates, the contamination profiles were species-dependent and differed from those observed in the soles. This indicated that trophic transfer is associated with a change of contamination profiles, further discussed in this communication. This study provides essential data to describe contamination levels and fate and, *in fine*, benthic habitat ecological quality in offshore systems.

### 3.09.P-Mo215 Understanding Mercury and Chlorinated Paraffins Biomagnification by Identifying Dietary Patterns in Killer Whales in Norway

**Carl Johan Ludwig Fagerlund<sup>1</sup>**, Eve Jourdain<sup>2</sup>, Clare Margaret Andvik<sup>3</sup>, Bo Yuan<sup>4</sup>, Anders Ruus<sup>5</sup> and Katrine Borga<sup>3</sup>, (1)Department of Biosciences, UiO, Norway, (2)Norwegian Orca Survey, Norway, (3)University of Oslo, Norway, (4)Stockholm University, Sweden, (5)Norwegian Institute for Water Research (NIVA), Norway

In Norway, the killer whale (*Orcinus orca*) could be considered a fish specialist, feeding mainly on Atlantic herring (*Clupea harengus*), however there has been increasing observational evidence for other prey types. Analysis of nitrogen and carbon isotopic ratios in skin of killer whales with known predation history further supported interindividual dietary variations in this population. Notably, while some killer whales appear to be fish-specialists, others also include marine mammals into their diet. Being an apex predator with long life span and thick blubber layer, the killer whales are vulnerable to biomagnifying pollutants such as methyl mercury (MeHg), especially when feeding on high trophic level mammal prey.

Our understanding of the dietary patterns of killer whales in Norway is far from complete. Specifically, the scope of predation on marine mammals is unknown, i.e., what proportion of killer whales in Norway include mammals in their diet and in which proportion. Seasonal switch between alternative fish resources is also poorly understood. As diet correlates with pollutant exposure, resolving dietary patterns, in parallel with studies of pollution is necessary to better understand pathways of contamination.

In this study, stable isotopes are analyzed in killer whale tissue samples as well as in known and other putative prey species with the aim of mapping the regional food web.

Bayesian statistical modeling will be used to estimate the diet composition of the killer whales based on stable isotopic nitrogen  $\delta^{15}\text{N}$  and carbon  $\delta^{13}\text{C}$  values, followed by an estimation of the trophic position of each investigated species and killer whales. Total mercury (Hg) concentrations up to 5.4  $\mu\text{g/g}$  d.w measured in killer whale skin have previously been reported. Additionally, persistent organic chemicals chlorinated paraffins (CPs), from very-short-chain to long-chain classes have been found in killer whale blubber and muscle tissue. Biomagnification of total Hg and CPs in killer whales, and in a selection of prey species, will be estimated and compared. There are still few studies of CPs biomagnification in marine food webs and wildlife. This study will add to our database and aid in gaining more insight into interindividual dietary variation in killer whales in Norway. Furthermore, including the multiple species in the food web combined with contaminant analysis will likely give a greater understanding of the behavior of Hg and CPs in the killer whale food web.

### 3.09.P-Mo216 Role of Marine Zooplankton in Long-Distance Transport of Pharmaceuticals From European Waters to the Arctic

**Ida Beathe Overjordet<sup>1</sup>**, Ewa Wielogórska<sup>1</sup>, Magda Caban<sup>2</sup>, Sławomir Kwaśniewski<sup>3</sup>, Dominika Walecka<sup>3</sup>, Ingrid Ellingsen<sup>1</sup>, Konstantinos Kotzakoulakis<sup>1</sup>, Antonio Sarno<sup>1</sup>, Roland Kallenborn<sup>4</sup>, Mari Creese<sup>1</sup>, Lisbet Sorensen<sup>1</sup>, Trond Nordtug<sup>1</sup>, Katarzyna Smolarz<sup>5</sup>, Adam Sokolowski<sup>2</sup> and Filip Spiralski<sup>2</sup>, (1)SINTEF, Norway, (2)University of Gdansk, Poland, (3)IO PAS, Poland, (4)Norwegian University of Life Sciences (NMBU), Norway, (5)Division of Marine Ecosystems Functioning, University of Gdansk, Poland

In recent years, several pharmaceuticals and personal care products (PPCPs) have been observed in marine environment as many of them do not decompose completely in sewage treatment. Some of these biologically active compounds have been shown to accumulate in or adsorb on living organisms. Marine organisms, such as zooplankton, may therefore act as vectors in long-distance transport of PPCPs from polluted coastal waters into pristine environments, such as the Arctic, where they can negatively affect marine biota. To investigate whether such transport occurs, we collected zooplankton from surface water layers at 20 sites on the route of northward transport of Atlantic water masses from the North Sea to the Fram Strait. Sampling sites were located in areas representing marine domains influenced by both coastal and Atlantic currents. After lyophilisation, samples were subjected to ultrasonic-assisted extraction in a mid-polar environment. Concentration of 36 commonly used PPCPs (including antibiotics, antidepressants, stimulants) and pesticides was measured using Liquid Chromatography – Mass Spectrometry (LC-MS).

Additional samples for determining zooplankton taxonomic composition and estimating the biomass at the sampling sites were collected and analysed to help explain potential patterns in PPCPs contents. In the research area the most abundant species were *Oithona similis* and *Calanus finmarchicus*, while zooplankton biomass was dominated by *C. finmarchicus* and *Parasagitta elegans* (in the North and Norwegian seas) or *Eukrohnia hamata* (Fram Strait region). Despite the dominance of the ubiquitous *Oithona similis* or widespread boreal species (*P. elegans*, *E. hamata*) in the species composition, demographic structure of *Calanus finmarchicus* suggests the presence of different biogeographical domains on the route of northward transport of Atlantic waters.

### 3.09.P-Mo217 Declining Concentrations of Chlorinated Paraffins in Endangered St. Lawrence Estuary Belugas (*Delphinapterus leucas*): Response to Regulations or a Change in Diet?

**Antoine Etienne Simond<sup>1</sup>**, Peter Ross<sup>2</sup>, Jory Cabrol<sup>3</sup>, Véronique Lesage<sup>3</sup>, Stephane Lair<sup>4</sup>, Million Woudneh<sup>5</sup>, Diwen Yang<sup>6</sup>, Hui Peng<sup>6</sup>, Katerina Colbourne<sup>7</sup> and Tanya Brown<sup>7</sup>, (1)Simon Fraser University, Canada, (2)Ocean Wise Conservation Association, Canada, (3)Maurice Lamontagne Institute, Fisheries and Oceans Canada, Canada, (4)University of Montreal, Canada, (5)SGS

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Very high levels of industrial contaminants in St. Lawrence Estuary (SLE) beluga whales represent one of the major threats to this population classified as endangered under the Species at Risk Act in Canada. Elevated concentrations of short-chained chlorinated paraffins (SCCPs) were recently reported in blubber of adult male SLE belugas. SCCPs are complex mixtures of alkanes of varying chain length (10 to 13 carbons) with varying degree of chlorine content (30 to 70% by mass) that have been used in North America primarily as lubricants and coolants in metal working fluids. SCCPs have the potential to biomagnify in marine food webs, and have been found to induce neurotoxic and endocrine disrupting effects in mammals and to potentially alter lipid and amino acid metabolisms in belugas. Recent regulations for SCCPs in North America, combined with their replacement by medium- (MCCPs) and long-chained chlorinated paraffins (LCCPs), highlight the importance of tracking this toxic chemical class. The objectives of this study were to evaluate (1) levels and profiles of chlorinated paraffins (CPs) in samples obtained from carcasses of adult male, adult female, juvenile, newborn, and fetus beluga, and (2) trends in adult male belugas between 1997 and 2018. Factors potentially influencing CP temporal trends such as age, feeding ecology and sampling year were also explored. SCCPs dominated (64 to 100%) total CP concentrations across all age and sex classes, MCCPs accounted for the remaining proportion of total CPs, and LCCPs were not detected in any sample. The chlorinated paraffin homolog that dominated the most in beluga blubber was C12Cl8. Adult male SCCP concentrations from this study were considerably lower (> 2000-fold) than those reported in 2020, likely reflecting a previously erroneous overestimate due to the lack of a suitable analytical method for SCCPs at the time. Both SCCPs and total CPs declined over time in adult males in our study (rate of 1.67 and 1.33% per year, respectively), presumably due in part to the implementation of regulations in 2012. However, there is a need to better understand the possible contribution of a changing diet to contaminant exposure, as stable isotopic ratios of carbon also changed over time.

### 3.10 Establishing the State-of-the-Science in Human Exposure to Micro- and Nanoplastic

#### 3.10.T-01 Secondary Nanoplastics Released from Food Packaging: Quantification and Formation Mechanisms

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Reliability of studies on micro(nano)plastics exposure becomes crucial to understand their potential hazard to environment and human health. However, analytical challenges limit our understanding of their formation and detection, thus hampering further investigation of their origins and effects. Plastic items are widely diffused in food packaging and they could potentially release secondary micro(nano)plastics during use in every-day life. So far, a validated and harmonized strategy to tackle this issue is not available. Here, we provide a combined approach to quantitatively and qualitatively detect the release of micro and especially nanoplastics in water matrixes and to estimate direct consumer exposure during common use of different food packagings.

We demonstrated that plastic breakdown to micro and nanoplastic can occur during the normal use of polyethylene (PE) sealing of drinking water bottles, rice cooking bags and ice-cube bags as well as of polyamide-6 teabags. In particular, PE cap sealing of the water bottles could release particles with a size distribution ranging from few hundred nanometers up to about one micron and we estimated a mass release to be in the order of few tenths of nanograms per opening/closing cycle. Additionally, the mechanical stress altered the physical-chemical characteristics of the generated nanoplastics and degraded their material properties, thus complicating the spectroscopic chemical identification. Furthermore, we highlighted the importance of using spectral databases that are not limited to just plastic materials, since released micro and nanoplastics from ice bags and rice cooking bags were contaminated with dyes and food residues. Finally, in the case of polyamide teabags, classical Fourier transform infrared (FT-IR) spectroscopy was used to measure the mass of released material. Here, temperature was shown to have a strong impact on the morphology and aggregation status of the released particles so posing scientists and legislators with a challenging question: should we consider keeping the heat-released material after cooling as being “oligomeric” or should we take into account the possible formation of nanoplastics?

#### 3.10.T-02 $\mu$ Raman Analysis of Airborne Indoor Microplastics down to 1 $\mu$ m – Should Facemasks be Recommended?

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Inhalation of microplastics (MPs) mainly occurs in indoor environments and may potentially represent the main source of intake. Most of the atmospheric MPs are fibers, probably released from synthetic clothes and textiles. Sources of MP fragments can be legion, for example building materials, abrasions of plastic products, landfilling, and waste incineration. Consequently, the MP morphology, mobility, and composition in indoor environments depend on the type of activity performed not only at the chosen location but also in its surroundings. There is the need for improved analytical methods for small (1–10  $\mu$ m) airborne MPs, to assess the potential risks associated with their introduction into the human organism. Moreover, several works recognized that airborne MPs may lead to the “fiber paradigm” (increased bioreactivity due to the fiber shape). Generally, the inhalation of airborne particles is driven by their aerodynamic equivalent diameter (AED), on which the so-called PM classification was based. In particular, the PM10 (AED <10  $\mu$ m) and PM2.5 (AED <2.5  $\mu$ m) particles can reportedly penetrate the low respiratory tract and PM2.5 was even found in the alveoli. The present work investigates the presence of indoor airborne MPs down to 1  $\mu$ m in four different locations (a workshop, an office, and two apartments), the effect of human activity on airborne MP abundance, and whether wearing facemasks would limit its intake.

Indoor air was sampled with a pump (~2 L/min) through a 1  $\mu$ m Silicon filters with 13 mm of diameter (Smart Membranes GmbH, Germany) hosted in a stainless steel sampling funnel (EMD Millipore Corporation, USA). Each sampling event took 24 hours and was repeated during a workday and a day in the weekend to account for the different levels of human activity in the sampling locations over time. Each sample was taken in triplicate: without facemask, with a surgical mask, and with an FFP2

mask mounted onto the inlet of the sampling funnel. The filters were then directly analyzed with Raman micro-spectroscopy (Horiba Sas, France). Moreover, the sub-micron fraction will be qualitatively explored with Atomic Force Microscopy (AFM) to assess the presence of nanoplastics (NPs), providing novel and valuable knowledge on indoor MP and NP pollution. The low-cost sampling method and the absence of sample preparation render the present method applicable to monitoring indoor environments with regard to human airborne MP intake.

### **3.10.T-03 What if You Eat Nanoplastic? An In Vitro Study of Nanoplastics' Gastrointestinal Digestion Using Model Food Matrices for Bioavailability Assessment**

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Nanoplastics, defined as plastic particles smaller than 1000 nm, remain one of the major environmental concerns. According to the current state of knowledge, macroplastic waste pervades into the environment and subsequently undergoes fragmentation to smaller fragments, reaching sizes of nanometer range. Due to their sizes, nanoplastics are known for their propensity to absorb in tissues of living organisms, from where they can be moved to food chain and eventually ingested by humans. There is evidence that nanoplastics have detrimental influence on living cells. However, the behaviour of plastic particles in conditions of human digestive tract has not yet been scrutinized, leaving a knowledge gap regarding the fate of nanoplastics between ingestion and absorption to human cells. Moreover, the influence of food matrix, with which particles are most probably ingested, on the risk of being absorbed, has not been investigated either.

To fulfill this gap, research was conducted on four model polymer nanoparticles: polystyrene of 50 nm and 200 nm, with and without surface functionalization by carboxyl groups, and on PLGA nanoparticles as a common biodegradable polymer. All particles were submitted to gastrointestinal digestion simulated *in vitro* by a two-step incubation with gastric and intestinal juice. After each step particles were analyzed by asymmetric flow-field flow fractionation with multi-angle lights scattering detection (AF4-MALS), which is a size-based separation technique. Additionally, for the largest particles, SEM measurements were conducted. The results showed that all polymer particles agglomerate during gastrointestinal digestion. The degree of agglomeration is, however, dependent on the particle characteristics.

We are now focusing on studying the influence of food matrix on the digestion process. For that, we carry out *in vitro* digestion and analysis as described above on polymer nanoparticles together with food products of high content of proteins, fats and complex sugars. We evaluate the impact of each of those substances separately and of the mixture of them, imitating an average meal. We hope to contribute in this way to the general knowledge on the hazards that nanoplastics may pose to the consumers.

### **3.10.T-04 Identifying Matrix Interferences in Analysis of Human Exposure to Polymers**

**Cassandra Rauert**, Nathan Charlton and Kevin Thomas, University of Queensland, Australia

Plastics pollution permeates all environmental compartments and whilst it is known that humans are constantly exposed to micro (<5 mm) and nanosized plastics (<1 mm), there are still large knowledge gaps on the extent of this exposure. The lack of current information on exposure and fate of polymers following exposure stems from challenges in the analysis of complex environmental matrices. Recently chemicals containing long chain hydrocarbons, such as stearates, have been identified as potential matrix interference in the analysis of polyethylene, providing false positive results. This study investigated the potential matrix effects from triacylglycerols (fatty acid type lipids) on analysis of polyethylene using pyrolysis gas chromatography mass spectrometry (Pyr-GCMS). A significant interference was observed from the three triacylglycerols tested (saturated, monounsaturated and polyunsaturated lipids). Subsequently, an effective enzyme digested method was developed to digest the triacylglycerols into smaller molecules that were removed using accelerated solvent extraction. The method was validated using food matrices (egg, milk, lamb), with excellent polyethylene recoveries from spiked samples (100-120%). This study presents for the first time an effective and optimised method for extraction and analysis of polyethylene from medium to high lipid content matrices and highlights the importance of assessing the matrix being extracted to improve confidence in reported polymer concentrations.

### **3.10.T-05 Micro- and Nanoplastic Quantification in Human Blood**

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The recent detection of micro and nano-plastic (MNP) particles in blood revealed that microplastics are taken up in the human body and transported via the bloodstream. These scientific findings received significant attention and impacted the understanding of human internal microplastic exposure. The toxicity of MNPs may be influenced by multiple physical characteristics such as shape, size, and polymer type and composition. The occurrence of MNPs in lung tissue supports human inhalation as a route of environmental exposure. In addition, MNPs were also reported in human breastmilk and human placenta, indicating that the unborn foetus and new-borns are exposed. The quantitation of MNPs in biological matrices and their possible effects and interactions are still poorly investigated, mainly due to the lack of robust methods with a high degree of sensitivity and selectivity. Pyrolysis-gas chromatography coupled to mass spectrometry (Py-GC-MS) has been successfully applied for the detection of several polymers in environmental samples. Using this destructive method, MNP particles (size range larger than 700 nm) were recently analysed in human whole blood samples. In this study, we present a robust and sensitive sample preparation and analytical method using Py-GC-MS. To ensure the accuracy of the analytical method, method validation was performed using an in-house quality control (QC) sample spiked with six polymers (polyvinyl chloride (PVC), poly(methyl methacrylate) (PMMA),

polypropylene (PP), polystyrene (PS), polyethylene (PE) and polyethylene terephthalate (PET)). The method was applied to measure plastic particles in 70 human whole blood samples from healthy volunteers. The QC samples were included in batch analysis to assess the reproducibility. The average recoveries for each polymer showed acceptable accuracy, ranging from 77 to 132%. Analysis done on blood samples (n=70) showed that PMMA, PP, PE, PET and PVC were identified and quantified. The method applied could extract, identify and quantify low concentrations of major polymers used in plastics. Suitable quality control measures should be included in Py-GC-MS analyses to assess the reproducibility and effective recovery of polymer particles. The results from this study have shown that plastic particles were detected in blood and are therefore bioavailable for uptake into the human system.

### 3.10.P Establishing the State-of-the-Science in Human Exposure to Micro- and Nanoplastic

#### 3.10.P-Th188 Establishing a Methodology for Analysis of Microplastics in Drinking Water in the European Union

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Food and drinking water are probably the most discussed sources of concern regarding human exposure to microplastics. Globally, microplastics have been shown to be present in water with sizes from a few  $\mu\text{m}$  to several mm. Reported concentrations typically range from an upper level of several hundred particle per litre to values which are often much less than the equivalent of 0.1 particle/litre. In the European Union (EU), the analysis of microplastic compositions indicates that about 90% of the particles are distributed amongst seven polymer types and concentrations are usually below 1 particle per litre - with the majority of samples being found to have concentrations significantly below 0.1 particle per litre. Even if the actual concentration levels of microplastics in drinking water across Europe seem to be low, the 2021 recast of the European Union's Drinking Water directive includes provisions for future monitoring of microplastics in domestic water supply chains. Consequently, the European Commission has the obligation to adopt a methodology for measuring microplastics in drinking water until January 2024. Establishing a methodology includes critical evaluation of already existing data on microplastics contamination in drinking water, careful consideration of the availability, capabilities and robustness of already existing sampling and analytical methods. The low levels reported by literature suggest that instrument sensitivity will be a major limiting factor in determining an appropriate methodology. Our presentation highlights some key input elements examined at the selection of the possible technological solutions from the basic choice between mass or number based concentration to the applicability for wide scale monitoring purposes. The poster also presents a range of options for future recommendations including the fundamental components of selected approach(es) as well as the suggestions for descriptors to be reported with the intention of discussing still plausible details with the experts of the field.

#### 3.10.P-Th189 Microplastics in UK Drinking Water; Implications for Human Exposure

*Muneera Faraj Almansoori, Mohamed Abou-Elwafa Abdallah and Stuart Harrad, University of Birmingham, United Kingdom*

Microplastics are microscopic particles less than 5 mm. There is an increased concern for public health as MPs have been found in tap and bottled water. These MPs cause adverse health effects because of their polymeric composition, additives, or other compounds or microorganisms adsorbing onto their surfaces. Few studies have detected MPs in bottled and tap water, although the current state of knowledge on MPs pollution in drinking water is still very limited. This study investigated the presence, concentrations, and size distribution of MPs in 85 tap water samples in the United Kingdom, as well as 85 samples of bottled UK from 16 different popular brands with various packaging. Sample preparation involved a vacuum filtration with filter membrane 0.2 $\mu\text{m}$  pore size, digital microscopic and  $\mu$ -FTIR using the PerkinElmer 400 Spotlight imaging system. The average MPs in TW was  $81 \pm 64$  MPs/L with minimum 6 MPs/L and maximum 415 MPs/L, with an average size of  $12.3 \pm 1.8 \mu\text{m}$ . For the BW in different types of packing, the average of MPs was  $51 \pm 19$  MPs/L with minimum and maximum of 12 MPs/L and 88 MPs/L. The average particle size was  $10.4 \pm 0.73 \mu\text{m}$ . The most frequently detected polymer types were PVC, PET in TW and in PVC, PP in BW. In terms of morphology, fragments and fibres were the most abundant MPs constituting 89% and 90% of MPs detected in tap water and bottled water, respectively. Using the average recommended daily water intake by the national academy of medicine USA, the corresponding average exposure of UK adults to MPs via drinking tap water was estimated at 189 and 138 MPs/day for men and women, respectively. Lactating and pregnant women, who consume more water, will be exposed to an average of 194 and 153 MPs/day. While infants 41 MPs/day, and toddlers 66 MPs/day, are exposed at lower levels due to less consumption of water. This lower daily exposure should be considered with caution, given their lower body weight, and incompletely developed immune/endocrine systems. Exposure through drinking bottled water is generally less than that from tap water, given the smaller number of MPs in bottled water. These emerging contaminants may come from the water source, the surrounding airborne environment, or the pipets that transfer the water. Our data provide clear evidence on the presence of MPs in UK bottled and tap water and raise concern over the potential adverse health impacts on the UK population from inadvertent exposure to MPs via drinking water.

#### 3.10.P-Th190 Food Packaging as a Source of Micro- and Nanoplastics in Food: A Systematic Evidence Map

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Plastic food packaging plays a central role in modern food supply enabling transportation, storage, and marketing. However, the normal use of food packaging, such as bottle opening, can lead to the abrasion of micro- and nanoplastics (MNPs) which have been detected in a variety of foods. And, humans ingest these MNPs with their foods and beverages which may lead to adverse

health impacts. To better understand food contact materials as MNP exposure source, the MNPs present in food and their origin (e.g., certain types of plastic food contact materials) need to be evaluated.

Therefore, we provide an overview of the available scientific evidence on MNPs that are generated by the use of plastic food packaging or other plastic food contact articles (e.g., food processing equipment) and are released into food. We compiled a systematic evidence map by searching PubMed, Web of Science, and ScienceDirect for relevant studies published until the end of 2022, screening them for eligibility, and extracting the data.

We present an openly available database that summarizes the current knowledge on food packaging as a source of MNPs into food. We provide an overview of the MNP quantities detected in food and their characteristics (e.g., size, polymer type), the food or food simulant type they were detected in, and the polymer and usage type (e.g., bottle, wrap, as well as single vs. repeat use) of the food contact articles the plastic particles originated from. Furthermore, we identify pertinent knowledge gaps and provide recommendations for further research. We also evaluate if the applied methods and experimental setups allow for robust evidence that the MNPs detected in food originated from a plastic food contact article. We propose a best-practice methodology for investigating the migration of MNPs from food packaging. Since MNPs are a novel type of contaminant originating from (plastic) food packaging, our methodological approach is inspired by the common, legally required practice to assess the migration of chemicals from food contact materials. Based on the outcome, we will address gaps and may recommend precautionary methods for industry and policy to reduce MNP exposure via the normal use of plastic food packaging.

Overall, our systematic evidence mapping can guide future research concerning study objects, methods, and ways of reporting.

### **3.10.P-Th191 Microplastics in White Shrimp from Traditional Markets in Semarang, Indonesia**

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Numerous studies have examined the presence of microplastics in various types of seafood. Moreover, some studies have observed that marketed seafood contains more microplastics compared to freshly harvested seafood. Investigating microplastic contamination in seafood circulating in markets is necessary as the majority of people buy seafood from traditional markets and the concentration of microplastics can change during transport from the production site to the market. White shrimp (*Litopenaeus vannamei*) is a seafood species that is widely available in Semarang, as Central Java province is one of the largest producers of white shrimp in Indonesia. The aim of this study was to investigate the concentrations and characteristics of microplastics in white shrimp collected from traditional markets in Semarang. White shrimp were therefore collected from six traditional markets and mixed and randomly selected up to 15 shrimp of similar size (7.5 – 10 cm) per market. After digestion with 30% H<sub>2</sub>O<sub>2</sub> 20:1 (v/w) and separation with a saturated NaCl solution (1.2 g/mL), the microplastic concentration and characteristics were determined under a light microscope and polymer identification was carried out with  $\mu$ -FTIR analysis. Microplastics (MP) were detected in all samples at concentrations ranging from  $1.20 \pm 0.98$  to  $2.98 \pm 2.21$  MP/g wet weight. MP concentrations in white shrimp were 5 to 17 times lower compared to shrimp from supermarkets in Singapore and 0.77 to 8.6 times lower than shrimp from polyculture ponds in central Thailand, but 3 to 17 times higher compared to white shrimp from several supermarkets in South Korea. Fragments were the most common MP morphotype found, ranging from 73% to 89%. This result differs from other studies that found film and fiber to predominate. Based on the size distribution, particles with a size range between 20 and 100  $\mu$ m predominate, i.e. 59-72%, but also MP particles > 100-1000  $\mu$ m in size were found in Semarang white shrimp (24-39%). Methyl cellulose is the most common particle in white shrimp (50.6%), followed by the polyethylene group (28.2%), rubber (11.8%) and polyethylene-polypropylene copolymer (7.1%). These results indicate that attention needs to be paid to the handling of white shrimp during transportation, distribution and presentation in the market.

### **3.10.P-Th192 Analysis of Microplastics in Various Foods and The Assessment of Aggregate Human Exposure via Food Consumption**

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Evidence of MPs occurring in humans has been recently demonstrated. The primary route of human exposure to MPs is through the consumption of contaminated food and water. However, the mass of microplastic exposure is not well defined, restricting the human health risk assessment. The transformation of microplastic counts into a mass value can be uncertain due to the unstandardized methods of calculation and analyzing microplastics in various food matrices. Therefore, this study proposes optimal analytical methods for eight types of food, including 90 products of salt, soy/fish sauce, seafood, seaweed, honey, beers, and beverages. Microplastics could originate from external sources such as manufacturing processes rather than from food. Subsequently, human exposure to microplastics of each food type was reported in terms of the number and mass of particles per week per person by simple calculation and Monte Carlo simulation. Geometric means and standard deviations of parameters were used to alleviate the influences of outliers in the data set, providing more reliable measurements. The determinations revealed that average adult Koreans likely ingest  $1.4 \times 10^{-4}$  and  $3.1 \times 10^{-4}$  g of MPs per week, respectively. The findings of this study extend the understanding of microplastic occurrence in food and contribute to the development of human health risk assessments.

### **3.10.P-Th193 Characterization and Quantification of Tire and Road Wear Particles and Microplastic Polymers in Air and Human Blood Samples**

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Micro and nano-polymer pollution can be found ubiquitously in all environmental compartments. To understand the human

exposure to tire and road wear particles (TRWP) and micro and nanoplastics (MNPs) from the air, a real case scenario experiment was run in 3 different locations in Utrecht (The Netherlands). Therefore, PM10 was sampled on quartz filters were sampled using a high-volume pump and human blood was obtained from 25 volunteers that participated in the study. The polymers measured in this study were polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polymethyl methacrylate (PMMA), polyethylene terephthalate (PET), polypropylene (PP), natural rubber (NR), and styrene butadiene rubber (SBR). For the analysis of the quartz filters, a sensitive method was developed for the characterization and quantification of rubber and microplastic polymers using sonication of the quartz filters followed by pyrolysis gas chromatography-mass spectrometry (Py-GC-MS). Quality assurance and quality control (QA/QC) parameters were assessed, including extraction efficiency (recovery), method specificity (pyrolysis temperature optimization and unique pyrolysates selection), and sensitivity (LOD/LOQ). Moreover, the method was validated using the 5 different polymers (PE, PP, PS, PVC, and PMMA) with a size range between 1 and 5  $\mu\text{m}$ . In addition, the method for the quantification and characterization of MNPs in human blood developed by Leslie et al. (2022) was adapted with some improvements for the analysis of TWRPs. For the validation, NR and SBR were used for the assessment of the same QA/QC parameters described above. By using these two methods, a total of 225 blood samples and 20 quartz filters will be analyzed to study human exposure to MNPs and TWRPs via the air. The aim is to quantify and characterize TWRPs and MNPs to understand the exposure and potential human health effects of plastic and rubber pollution between an urban park, a busy traffic road, and a road near a roundabout.

### 3.10.P-Th194 MPs In the Indoor Air: Implications for Human Exposure

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Microplastics (MPs) are a group of emerging contaminants that have attracted increasing scientific and societal attention over the past decade due to their ubiquitous detection in all environmental compartments. So far, most studies on MPs focus on characterizing their occurrence, fate, and impact in the aquatic environment. Very little is known about the magnitude, patterns, and associated risks of human exposure to MPs, particularly indoors. Airborne MPs may cause adverse effects on human health through inhalation, including respiratory, immune and reproductive toxicity, according to the Agency for Toxic Substances and Disease Registry (ATSDR). Critical evaluation of the existing literature revealed the presence of MPs at higher concentrations in indoor air (from homes and offices) compared to outdoors. This raises concern given that people spend most of their time indoors. This study applied a combination of active and passive air sampling techniques to investigate the abundances, morphology, and composition of MPs in indoor air samples collected from homes ( $n=30$ ) and work placement ( $n=30$ ) in Birmingham, UK. MPs analysis was conducted using Perkin-Elmer spotlight 400 imaging system. Average concentration of MPs in the active air samples was  $16.2 \pm 5.1$  MPs/ $\text{m}^3$  in homes and  $13.1 \pm 6.7$  MPs/ $\text{m}^3$  in offices. For atmospheric deposition samples (passive sampling), the average MPs concentrations were  $3464.3 \pm 1464.2$  MPs/ $\text{m}^2/\text{day}$  in homes and  $4334.2 \pm 2462.5$  MPs/ $\text{m}^2/\text{day}$  in offices. In terms of morphology, various shapes of MPs (e.g., fiber, foam, and fragments) were identified, with fibres and fragments constituting 90% of the identified MPs in all studied samples. The average MPs size (on the longest dimension) in active air samples was  $10.65 \pm 5.55$   $\mu\text{m}$ , while much larger particles were detected in atmospheric deposition samples with an average size of  $21.13 \pm 10.62$   $\mu\text{m}$ . A broad range of polymer types was identified including PP, PE, PET and PS as the most abundant polymers. Using our active air sampling data combined with inhalation rates from USEPA exposure factors handbook, the average daily inhalation exposure of UK adults and toddlers at home was estimated at  $3.1 \pm 0.98$  and  $4.4 \pm 1.4$  MPs/kg body weight/day, respectively.

### 3.10.P-Th195 Method of Deriving Exposure Factor for Exposure Assessment of Microplastics During Activities in the Living Environment

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Microplastics have recently been recognized as a serious environmental problem, and interest in microplastics is increasing worldwide. Research related to the exposure assessment of microplastics in the living environment is currently insufficient, and it is difficult to acquire the exposure assessment methodology and applicable exposure factor of microplastics. Therefore, in this study, a data survey study was conducted to obtain information available for assessing microplastic exposure during activities in the living environment, such as exposure factor, exposure route, and exposure scenarios. Among household chemical products, laundry detergents, cleaning agents, microfibers, and disposable masks are products that are likely to cause microplastic exposure to humans and the environment, so they were selected as the products to be investigated in this study. A survey was conducted on the selected products to derive exposure factors. The purpose of the survey was to set the exposure scenario, derive the exposure factor, and obtain the necessary information to set the exposure assessment model, and the survey was conducted by dividing the questionnaire according to the characteristics of each target product. In the case of masks, exposure may vary depending on the material (KF94, KF80, dental mask, etc.) and shape (bird's beak type, dome type, etc.), and in the case of microfibers, it is necessary to consider whether a dryer is used or not, so a differentiated survey was conducted for each product. The exposure factor derived from this study is expected to be used as basic data for preparing exposure assessment techniques and performing risk assessment for household chemical products such as cleaning agents and laundry detergents, and products of social interest, such as textiles and masks.

### 3.10.P-Th196 A New Route of Exposure for Microplastics: Contact Lenses

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Humans are exposed to microplastics due to their various physical and chemical properties such as small size, surface area, and strong biological penetration ability to reach and penetrate various organs including the placenta and brain. Contact lens and lens cleaning solutions have been investigated as a source of microplastics that may cause this exposure. Within the scope of the study, a lens solution used daily for one month was sampled from a volunteer lens wearer. Daily exposure to microplastics in the used lens solution was analyzed. The used lens solutions collected daily were collected in 5 ml vials. An appropriate procedure was applied to the lens solution. Afterward, the solutions were visually analyzed using a stereo microscope and imaging with Image J software. Color, size, and shape analysis was performed for the used lens solution of each day and the chemical characterization was performed after visual analysis. In addition, a questionnaire was applied in which the volunteer talked about his daily activities. The color and polymer type of personal belongings (towels, bed linen, clothes, etc.) containing polymers that they used in their daily activities, which could be a possible source, were recorded in the survey. The results of the survey and the analysis results of the lens solution were correlated with each other. The widespread presence of MPs in lens solutions has confirmed and provides the foundation for future studies into understanding the human exposure pathways.

### 3.10.P-Th197 *In vitro* Bioaccessibility and Human Dermal Exposure Assessment of Brominated Flame Retardants in Microplastics

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Human exposure to microplastics (MP) is currently understood to occur via three distinct pathways including ingestion, inhalation and dermal contact occasioned by the presence of MPs in consumer products, dusts, water, air and foodstuff. Evidence of health effects associated with direct exposure to pristine MPs are lacking, however, there is an enormous concern over the toxicity of plastic additives (e.g. flame retardants (FR), per and polyfluoroalkyl substances (PFAS), phthalates etc.) released from MPs upon contact with human.

In this study, we optimized an *In vitro* physiological based extraction test by using synthetic sweat/sebum mixture (SSSM) to provide new understandings into the dermal bioaccessibility of various FRs from two MP standard reference materials (ERM 590 and ERM 591) and a polystyrene with known concentrations of FRs. The bioaccessible fractions of brominated flame retardants in these MPs were used to quantify their dermal exposure dose. All the studied BFRs were bioaccessible to varying degree. The daily exposure dose ranged from 0.05 – 22.34 ngkg<sup>-1</sup>bw d<sup>-1</sup> and 0.001 – 0.37 ngkg<sup>-1</sup>bw d<sup>-1</sup> for high and low MP intakes, respectively. While for toddlers, they ranged from 0.5 – 230 ngkg<sup>-1</sup>bw d<sup>-1</sup> and 0.01 – 3.85 ngkg<sup>-1</sup>bw d<sup>-1</sup> respectively for high and low MP intakes. For HBCDDs, the DED ranged from 1.13 – 6.27 ngkg<sup>-1</sup>bw d<sup>-1</sup> and 0.02 – 0.10 ngkg<sup>-1</sup>bw d<sup>-1</sup> for dermally exposed adult, while for toddlers, it ranged from 11.80 – 65.10 ngkg<sup>-1</sup>bw d<sup>-1</sup> for the high and low exposure scenarios, respectively.

### 3.10.P-Th198 A Comparative Study of Digestion Methods for Qualitative and Quantitative Analysis of Microplastics in Animal Tissues

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Microplastic (MP) pollution is ubiquitous in air, water, and soil, and contaminates the food chain. MP ingestion in animals and presence in human stool has been reported. This raises concerns about their potential absorption, distribution, and elimination *in vivo* and their health effects, but to obtain scientific data on this, it is first necessary to establish suitable analytical methods. This often involves either concentrating the sample on a filter and performing qualitative analysis using Raman or Fourier Transform-Infrared micro-spectroscopy, or solvent-extracting the sample for qualitative/quantitative analysis using pyrolysis-Gas

Chromatography Mass Spectrometry. The MP accumulated in animal and human tissues are hypothesised to be smaller and in more trace amounts than those in environmental samples. This presents challenges in the separation of MP from different tissues, such as muscle or fat, the need for lower detection limits, and background contamination prevention. This study aims to overcome these challenges, by optimising tissue digestion methods for the separation of MP from organic matter.

H<sub>2</sub>O<sub>2</sub>, Fenton's reagent, 1M-KOH and 5M-KOH were selected from the literature as the agents to test. Control and MP-spiked mock samples (n=3, beef steak, lamb liver) were prepared and used to (1) compare digestion efficiency (% weight reduction), (2) compare MP recovery (% weight reduction), and (3) observe impacts on MP (observing changes in colour, size, and shape). For MP-spiked mock samples, a known amount of MP (PS, PP, PE, PA or PVC) was mixed into the minced meat before forming into pellets and freezing.

The digestion efficiencies of 30%-H<sub>2</sub>O<sub>2</sub>, Fenton's reagent, 1M-KOH and 5M-KOH were 80.1%, 88.4%, 97.1% and 95.6%, respectively. For performance, operational safety and Green Chemistry principles, 1M-KOH is preferred. The overall average recoveries of PS, PP, PE, PA and PVC (n=3) was 98%. No physical change was visibly observed when exposed to 5M-KOH. Digestion efficiency, MP recovery and the agent's impacts on MP were obtained and discussed to determine the most suitable digestion method for the analysis of MP in animal tissue. It was concluded that 1M-KOH is the best digestion method. Future plans include a more in-depth investigation of the impact of tissue type (muscle, fat, or organ) on digestion efficiency.

### 3.10.P-Th199 An Enzymatic Digestion Protocol for the Characterization of Micro- and Nanoplastics in Maternal and Fetal Tissues

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Plastic can break down into smaller particles, called microplastics (<1 mm) and nanoplastics (<1 µm) (MNPs). The fate of these particles and their effect on human and animal health is still mostly unknown. Studies show environmental pollutants may have

an adverse health effect on the developing fetus, which makes the human placenta an important matrix in researching MNPs' health effects.

We focus on digestion protocols for placenta tissue to enable in-depth characterization of MNPs present in these samples. We aim for combining mass-based analysis with (spectro)microscopic techniques, to determine particle mass, number, morphology and chemical composition. To enable MNP characterization, the biological matrix has to be pre-processed to filter out components interfering with analysis. A successful digestion needs to sufficiently remove biological matrix, while assuring stability of MNPs in terms of chemistry, morphology, and particle number. To assess success of the digestion method and subsequent analysis, samples were spiked with a known concentration of well-defined commercial MNPs, namely fluorescent polystyrene (FPS) spheres.

1g of placenta tissue with known concentrations of FPS MNPs was digested with five different enzymes combined with three different buffers. Digestion was followed by filtration over a 700 nm pore size glass fiber filter. The amount of tissue left on the filter and the color of the filter were used as indicators for the success of the digestion. The obtained liquids were filtered over 90 nm pore size alumina filters which were analyzed with Confocal Fluorescence Microscopy (CFM) and Scanning Electron Microscopy (SEM). Our first results show that FPS MNPs were recovered from digested samples with no visible changes in morphology.

An enzymatic digestion protocol has been tested and optimized for the characterization of FPS MNPs in human placental tissue. At this stage Proteinase K in TRIS-HCl, pancreatin in TRIS-HCl and trypsin in PBS were found most suitable for tissue digestion. PS MNPs were found back after digestion and remained stable under the conditions used in this protocol. Our next steps will address recovery rates to further establish suitable digestion protocols for both mass-based and imaging analytical methods. This development in digestion methods for complex matrices is an important step in creating a risk assessment of MNPs to the unborn child.

### **3.10.P-Th200 Progress Towards Accurate Detection, Characterization, and Exposure Assessment of Micro- and Nanoplastics to Support Risk Assessments for Early-Life Health Within the Aurora Project**

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Being able to accurately detect and characterize micro- and nanoplastics (MNPs) is a crucial first step in assessing human exposure to MNPs and their potential impacts on early-life health—which is the goal of our five-year, EU-funded Horizon 2020 research project, AURORA. However, detecting and characterizing MNPs, especially nanoplastics (NPs), in complex human tissues comes with significant analytical challenges. NPs are too small for light-based microscopy such as UV, IR, or visible (spectro-) microscopy. Besides their small size, the weight-per-particle decreases dramatically from micro to nanoplastics, making mass-based characterization techniques difficult even with (relatively) larger particle numbers. To complicate the situation further, NPs primarily consist of carbon, hydrogen, and oxygen, which makes them chemically similar to the organic matter that surrounds them in tissue samples limiting the use of imaging methods based on element contrast or spectroscopy methods. To enable better characterization and exposure assessment of MNPs in complex tissue samples, including human placentas and blood, we are developing new sampling and analysis standards that combine approaches to consider both quantitative and qualitative information by using both mass-based and numerical techniques. We have combined several micro-spectroscopic techniques that go beyond the diffraction limit, including for example confocal fluorescence microscopy, atomic force microscopy (AFM) derived techniques like AFM-IR, or quantitative nanomechanical mapping. Another approach is correlative imaging such as scanning electron microscopy combined with Raman or IR micro-spectroscopy. In addition, we have optimized sample preparation using digestion protocols to remove natural organic matter, thereby ensuring that the MNPs do not change chemically or morphologically. Efforts are also being made to determine recovery rates of the sample preparation techniques. In the AURORA project, we are advancing analytical methods to detect NPs, characterize their physical-chemical properties, and assess mass-based levels in European birth cohort samples. These methodological advances will allow for improved quantitative assessments of exposure during pregnancy and early life as well as potential associated risks.

### **3.10.P-Th201 Chemicals in Medical Plastics**

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The healthcare sector has a primary focus to ensure that patients receive quality and comprehensive care. However, research is beginning to suggest that the healthcare sectors reliance on single use plastics challenges that goal. Plastic products are highly favorable in society for their wide range of application and convenience and specifically within healthcare for the ability of products to arrive sterilized and ready for use. However, the versatility of plastic products is the result of additives, plasticizers, and chemicals that are added to the polymers to obtain the desired qualities. Studies have shown that some chemicals, such as BPA (bisphenol-a), can leach from the plastic product and affect the consumers of the product. The effects of these chemicals, including BPA and others, have been proven to range from reproductive impairments to the development of cancer. Furthermore, these chemicals have the potential to be released into the environment at the end-of-life phase of the product. When considering



the healthcare sector and their reliance of single use plastics specifically, the concern of leaching chemicals to vulnerable groups of patients becomes high. Additionally, when considering the recyclability of these products, it becomes paramount to consider the substitution of these chemicals to ensure the circularity of polymers within the value chain. In this comprehensive literature review, chemicals such as BPA, PVC, and PFAs which are commonly found in plastic products will be addressed with a specific focus on healthcare products and the potential affect these chemicals can pose on human health. Specific products will be identified and recommendations to phase out the use of these chemicals to producers of medical products and devices will be addressed. At last, in line with the circular economy action plan and upcoming UN treaty on plastics, the policy proposal of removing or phasing out these chemicals in plastic products will be suggested as a main focus.

### 3.10.V Establishing the State-of-the-Science in Human Exposure to Micro- and Nanoplastic

#### 3.10.V-01 Development of Analytical Methods for Detection of Nanoplastics in Fish

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Due to the mismanagement of the corresponding waste, plastic pollution is so ubiquitous and pervasive that it has become a threat to the environment and human health. The slowly degrading/fragmenting plastic debris forms a highly heterogeneous group of particles with different sizes (i.e., from centimeter over millimeter and micrometer to nanometer scale), shapes, densities, and chemical compositions. Detection, quantification and source identification of nanoplastics (NPs, 1-1000nm) in different matrices is still one of the largest challenges in this context due to the lack of adequate sample preparation procedures and straightforward analytical techniques for their characterization and quantification. Taking into account that food is one of the major exposure routes of NPs for humans and the potential risk for human health, it is of crucial importance that appropriate analytical methods for the analysis of NPs in food are developed.

In the framework of the EU-funded research project MS4Plastics (H2020-MSCA-IF-2020 - Grant Agreement No 101023205) different extraction protocols were tested to isolate NPs (polystyrene standards with particle size of 60, 90, 150, 240, 500 or 1000nm) from the fish muscle tissues (skin removed) of specimens of European eel (*Anguilla anguilla*) sampled in Flemish freshwaters. Several approaches including (1) acid digestion, (2) alkaline digestion, (3) solvent extraction and/or (4) enzymatic digestion were tested to investigate whether the developed extraction protocols could affect the NPs size that was measured by using dynamic light scattering. Finally, the possibility of using (1) asymmetrical flow field-flow fractionation (AF4) hyphenated to ultraviolet-visible spectroscopy (UV/VIS) and multi-angle laser light scattering (MALLS) and (2) pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) as tools for characterization of isolated NPs were investigated.

The state of the art of the sample preparation methods selected with detection of NPs by AF4-UV/VIS-MALLS and Py-GC/MS will be presented, their performance will be evaluated, and the challenges that had to be addressed will be discussed.

#### 3.10.V-02 Uptake and Distribution of Polyisobutylene Microplastic in the Gut of Zebrafish Following Dietary Exposure

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Polyisobutylene (PIB) is a kind of elastomer that has excellent properties in terms of gas and moisture impermeance, damping, chemical and oxidative stability, and so on. Because of its great flexibility at room temperature, resistance to heat, and capacity to transmit gases, it finds widespread application in a number of industrial settings. These include tire inner liners and tubes, sealants, adhesives, condenser caps, pharmaceutical stoppers, and chewing gums. Although PIB biodegrades in water, the effect of its micro-size on toxicity has not been investigated. Here, we assessed the toxicity and accumulation of PIB using a zebrafish model and synthesized and characterized micro-size PIB. The zebrafish has several desirable characteristics as a study model, including its short generation time, optical transparency, high reproductive rate, and high ability to adapt to new environments, a high-quality vertebrate model for toxicology studies. We synthesized pristine and fluorescence PIB-microplastic (MP) with particle sizes of ~2-5µm using the solvent evaporation technique. During a 21-day feeding study, adult zebrafish were given food containing either  $2.7 \times 10^2$  or  $5.2 \times 10^2$  PIB-MP particles per liter. Within 21 days, PIB-MP accumulated in the fish gut, and the histopathology data demonstrate that this accumulation of PIB-MP in all the gut areas resulted in the aberrant architecture of the GIT. Interestingly, an analysis of fish behavior revealed a remarkable increase in their aggressiveness and pronounced alteration in the locomotor activity of the exposed groups. Evaluation of the metabolic changes caused by PIB-MP and comprehensive characterization of gut microbial compositions in zebrafish exposed to PIB-MP is ongoing but preliminary studies suggest that the distribution of these particles in the gut could alter the metabolites resulting in microbiota dysbiosis. However, further study is required to validate this, which we will present at the meeting.

### 3.11 Fate of Organic Contaminants in the Soil-Plant Continuum – Coupled Processes and Appraisal of Potential Impacts and Risks

#### 3.11.T-01 Investigation of Uptake and Translocation of Plant Protection Agents by Crops via HPLC-HR-MS and MALDI-MSI

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Knowledge about the uptake and translocation, as well as metabolization of pesticides in crops is of great importance to human health and sustainable plant protection. Due to differences in experimental setups of plant uptake studies, such as varying growth

stages, composition of nutrient solution and other ambient influences, a comparison of uptake between different plants or plant protection agents often proves difficult. We established a simple and comparable hydroponic plant uptake study design and developed a suitable multi-component extraction method resulting in high recoveries while showing low matrix interference. We conducted several hydroponic plant uptake experiments to gain knowledge about the behavior of six physicochemically different pesticides, namely chlorpyrifos, tebuconazole, metalaxyl-m, fluopyram, spirotetramat and imidacloprid, taken up by maize and other important crops. Experiments at well defined BBCH were conducted in a plant chamber which enables constant and reproducible ambient parameters like light intensity, light duration, temperature and relative humidity. After harvesting, plant material was freeze dried, crushed and homogenized. Highly selective and sensitive HPLC-HR-MS<sup>n</sup> was used for unambiguous identification and quantitation of initial compounds and their metabolites in roots, stem and leaves. MALDI-MSI at high spatial resolution allows precise localization of target compounds and insights into mechanistic processes inside the plant. We report new findings on the plant uptake of the pesticides in correlation with their physicochemical parameters, keeping a set of key parameters constant during the uptake experiments.

### 3.11.T-02 Contaminants of Emerging Concern in the Reclaimed Wastewater- Soil- Plant Continuum

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Reclaimed wastewater irrigation has become a common agricultural practice in water-scarce regions. This practice exposes the agricultural environment to a wide range of contaminants emerging concerns (CECs). Once in the soil, CECs may undergo several processes such as degradation (mainly biodegradation), sorption to soil particles which is affected by the CEC's physicochemical properties and the soil organic matter content, and plant uptake. Generally, non-ionic and relatively lipophilic CECs can passively cross the root semipermeable membrane, whereas charged CECs may interact with the negatively charged membrane and as a consequence, their uptake would be hindered. CEC translocation from root to shoot in the xylem depends on its solubility in water and transpiration stream. In the current study, we used data collected from 445 fields irrigated with reclaimed wastewater to provide comprehensive insights regarding the fate of CECs in the irrigation water-soil-plant continuum. Leafy vegetables, carrots, oranges, tangerines, tomatoes, potatoes, avocados, and bananas were sampled in the study and are representative of the various plant parts. All irrigation water, soil, and plant samples were contaminated with CECs between 4-46, 1-21, and 0-13 analytes per sample, for the different media respectively. The reduction in the occurrence of CECs in the soils as compared to irrigation water was probably a result of rapid biodegradation in the soils. The further reduction in the occurrence of CECs in crops was a result of the semipermeable root membrane which acted as a soil-plant barrier.

Irrigation water post-soil-aquifer treatment exhibited the lowest occurrence and concentrations of CECs as compared to other wastewater treatments. These results are attributed to the long residence time in the soil aquifer and the infiltration through the sand. This trend continued to the soils and the irrigated plants, indicating the importance of the CECs concentration in the irrigation water. Within the plant, leaves exhibited the highest CECs occurrence and concentration. This results from the passive uptake of CECs into the plant roots and translocation towards the leaves via the xylem with the water stream. Our data suggest that the soil acts as a reservoir for persistent CECs which retain and accumulate in the irrigated soils. These CECs may be released to the soil solution during the rainy season or between irrigations and be bioavailable for plant uptake.

### 3.11.T-03 Effect of Irrigation Alternation on the Accumulation of Contaminants of Emerging Concern in Common Vegetables

**Qingyang Shi and Jay Gan, Department of Environmental Sciences, University of California, Riverside**

Recycled water has been regarded as a promising alternative water resource to alleviate water scarcity in agriculture. However, the potential risk of accumulation of contaminants of emerging concern (CECs) in plant tissues due to recycled water irrigation hinders the acceptance and adoption of such reuse. In the present greenhouse study, we propose a hybrid irrigation scheme as a means to reduce CEC accumulation in plants. The concentration of 11 commonly occurring CECs in plant tissue in different growth stages was compared between two treatment sets: one group received CEC-spiked water for the whole growth period; the other group was only exposed to CECs for half of the period and was switched to clean water irrigation afterwards. Three types of vegetable were cultivated, including radish, lettuce and tomato, representing a root, leafy and fruit vegetable, respectively. It may be expected that alternating to freshwater for the second half of the growing season could significantly reduce CEC accumulation in the final edible produce, as time is allotted for extensive metabolism and growth dilution to eliminate CECs. Through evaluating a practice that aims to reduce the accumulation in the edible part of vegetables with a simple modification, findings from this study may minimize human exposure risk, promote public awareness of water reuse practices, and improve the safe reuse of marginal waters.

### 3.11.T-04 A Novel Modelling Approach to Prioritise Pharmaceuticals with an Accumulative Potential Within Crops - A Wastewater Irrigation Scenario

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Wastewater irrigation creates a route for human pharmaceuticals to reach agricultural soils, although very little is known regarding the potential for numerous human pharmaceuticals to accumulate in arable crops with experimental studies primarily focussing on a handful of chemicals. To bridge this knowledge gap a novel modelling approach was developed which utilised high resolution mass spectrometry datasets to define which pharmaceuticals are potentially present in wastewater irrigation following their detection in effluents. In total 178 pharmaceuticals were evaluated across various processes from, consumption –

wastewater treatment plant (WWTP) - irrigation – plant accumulation. In a first step physiochemical properties and chemical fate parameters (degradation and sorption in soil and sludge) were predicted, the outputs of which were then used within our defined framework comprising of 1. Activity SimpleTreat (WWTP fate), 2.  $PEC_{soil}$  (wastewater irrigation), and 3. a plant uptake model for *Zea mays*. The modelling framework was applied to evaluate three wastewater irrigation scenarios from regions with high wastewater reuse (Israel, Australia, and California). The top ten pharmaceuticals that were accumulated in *Zea mays* root, shoot, and fruit following wastewater irrigation were, piracetam > primidone > carbamazepine > mycophenolic acid > levetiracetam > warfarin > captopril > sulindac > acetaminophen > losartan (> 1.5 mg/kg FW). Factors affecting pharmaceutical accumulation were, consumption, degradation (sludge and soils), sorption, and the octanol-water partition coefficient. A compound specific effect was observed with regards to soil properties and pharmaceutical accumulation in plant. For example, between pH's of 5.4 and 7.7 the total primidone accumulation varied between 4.65 and 6.88 mg/kg (FW) respectively, whereas for warfarin little differences were observed (1.68 and 1.78 mg/kg). Our results highlight where future research efforts need to focus in terms of chemicals of interest which are currently not research priorities (e.g., piracetam). The human environmental risk assessment does not consider non-potable sources for pharmaceuticals to reach the environment, which is a problematic given that this process is increasing and has greater rate of application than that of sludge/manure applications. Therefore, the presented methods will be beneficial in building a regulatory framework as well as the benefits that arise from fate screening.

### 3.11.T-05 A Novel Mechanistic Model to Describe the Fate of Chemicals in the Soil-plant Continuum

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Plants represent the most common pathway into the human food chain for environmental pollutants. Chemicals are taken up by plant roots from the soil and then translocated towards the edible parts, where they are bioaccumulated or eventually metabolized. These transport mechanisms are driven by the physicochemical interaction between soil, plant, and atmosphere. It is thus important to develop numerical tools that are able to accurately predict the fate of contaminants in this complex domain. With this in mind, in a series of recent studies, we developed a numerical framework that couples the widely-used model HYDRUS with the multi-compartment dynamic plant uptake routine. The resulting model mechanistically describes water flow, root uptake, and reactive solute transport in the soil, and is able to predict the fate of neutral and ionizable compounds in multiple plant compartments. The model has been successfully calibrated and validated against experimental data on the translocation and transformation of neutral (carbamazepine and its metabolite carbamazepine 10,11-epoxide) and ionic (citalopram, fexofenadine, irbesartan, sulfamethoxazole, clindamycin and its metabolite clindamycin sulfoxide) compounds in green pea plants. To favor its widespread adoption, the model has been coupled with a graphical user interface and included in the HYDRUS software suite. In this study, we provide an overview of the model structure, and report some of the most meaningful experimental scenarios that demonstrate its predictive capabilities.

### 3.11.P Fate of Organic Contaminants in the Soil-Plant Continuum – Coupled Processes and Appraisal of Potential Impacts and Risks

#### 3.11.P-Mo218 Uptake of Pharmaceuticals into Edible Crops: A Field Case Study

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Irrigation with treated wastewater has been shown to introduce pharmaceutical and personal care products (PPCPs) into the environment. Further, use of treated wastewater in agriculture can lead to soil contamination with PPCPs and their metabolites, providing a route for accumulation in edible crops. Despite this, limited knowledge exists on the risks that irrigation of treated wastewater poses to non-target organisms and human health. Accordingly, the aim of this study is to determine the number, type and concentrations of pharmaceuticals and personal care products (PPCPs) in edible crops in market gardens and pasture in Victoria, Australia. Specifically, this study has four specific aims: (1) determine concentration of pharmaceuticals in irrigation water and soil; (2) quantify the concentration of pharmaceuticals in crops for human and animal consumption (florets, leaves, root) irrigated with A and B class irrigated water; (3) investigate the presence and potential of pharmaceuticals to bioaccumulate in plants subject to irrigated water; (4) assess the associated potential risk to human health through consumption of edible crops irrigated with treated wastewater. The study analyses samples from two different recycled water irrigation scenarios: (1) class A market garden crops; (2) class B-C pasture for livestock grazing in Victoria. For both scenarios, two farms will be selected along with a reference site (total of 6 sites: 2 market gardens, 2 pastures for livestock grazing, 2 reference sites). The selected reference farms occur in the same geographical region as the irrigated sites and grow the same crop type as the scenario farms but without recycled water. For both scenarios (market garden & pasture), 10 crops are collected from each site. From each plant, its leaves, floret, and roots will be analysed for PPCPs. In addition, 10 surface soil samples and 6 water samples (pre- and post-irrigation) will be analysed for PPCPs at each site. In total, 30 water, 138 plant, and 69 soil samples will be analysed. This will be one of the first studies investigating uptake of pharmaceuticals and personal care products into edible crops and pastures in Victoria (Australia), and we will be presenting the study in full detail in the conference.

#### 3.11.P-Mo219 Mobility of Selected Micropollutants in Agricultural Soils of the Czech Republic

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The sorption of organic contaminants in soils and sediment is a crucial factor affecting their mobility in the vadose zone environment. The Freundlich sorption isotherms were evaluated for eleven micropollutants and eight soils. The highest Freundlich sorption coefficients,  $K_F$ , were obtained for triclosan ( $324 \pm 153 \text{ cm}^3/\text{n}\mu\text{g}^{1-1/\text{n}}\text{g}^{-1}$ ) followed by sertraline ( $120 \pm 74$ ), venlafaxine ( $74.3 \pm 41.2$ ), telmisartan ( $33.3 \pm 13.6$ ), atorvastatin ( $8.66 \pm 4.78$ ), bisphenol S ( $8.03 \pm 4.87$ ), lamotrigine ( $6.92 \pm 3.02$ ), 2-phenyl benzimidazole-5-sulfonic acid ( $3.77 \pm 2.25$ ), memantine ( $3.42 \pm 1.64$ ), 1-methyl-1H-benzotriazole ( $2.05 \pm 0.99$ ), and valsartan ( $0.88 \pm 0.89$ ). The  $K_F$  values for the individual compounds were correlated with soil properties. Multiple linear regressions were used to derive equations for predicting the  $K_F$  values using the soil properties included in the map of agricultural soils of the Czech Republic. These equations always indicated positive correlations with oxidizable organic carbon and clay content. They also included either a negative or positive correlation with  $\text{pH}_{\text{KCL}}$ . A positive correlation with  $\text{pH}_{\text{KCL}}$  was obtained for venlafaxine, memantine, and sertraline, which were mostly positively charged. A negative correlation with  $\text{pH}_{\text{KCL}}$  was obtained for the remaining compounds. These equations, the soil map, and the database of soil properties were used to predict the  $K_F$  value distributions within the Czech agricultural soils. It resulted in similar  $K_F$  distributions' patterns for valsartan, lamotrigine, atorvastatin, and telmisartan (with a positive correlation between  $K_F$  and hydrolytic acidity), which considerably differed from the  $K_F$  patterns for the other compounds (with a positive correlation between  $K_F$  and base cation saturation or organic carbon content). These maps were used to delineate areas with a leaching potential of the compounds towards groundwater that will serve as a tool for assessing a potential groundwater vulnerability. This information will be used to improve the monitoring of groundwater quality within the Czech Republic, to identify areas unsuitable for broader utilization of wastewater for irrigation or stabilized sludge as a soil amendment, or to propose the safe application of these products onto agricultural soils.

### **3.11.P-Mo220 The Behavior of Sertraline in Soil-plant System**

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The behavior of sertraline in soils and plants has been investigated in several of our studies. In the framework of these studies, contradictory results were obtained. It has been discovered that this compounds sorbs very strongly in soils because the sorption of this compound, which occurs in soils predominantly in a cationic form, is mainly driven by the ionic bonding onto the negatively charged surface of soil constituents. The average Freundlich sorption coefficient in 8 soils was  $120 \pm 74 \text{ cm}^3/\text{n}\mu\text{g}^{1-1/\text{n}}\text{g}^{-1}$ . Another study showed that desorption of this compound, which frequently occurs in sewage sludge from wastewater treatment plants, from this material is very low (i.e., desorbed fraction < 1%). This finding is consistent with previous observations. On the other hand, it has also been found that in some soils, as Chernozems developed on loess, this compound can be easily taken up by plants (in this case spinach), accumulated in roots and even in plant leaves, contrary to its considerably lower uptake and low accumulation in plants planted in Cambisols. Finally, our recent study showed that despite its expected high sorption in soils and low desorption rate from sewage sludge, this compound could leach from soil beds amended with sewage sludge or composted sewage sludge, which were exposed to natural climatic conditions and drip irrigation. We assume that the binding of this compound to dissolved organic matter and/or the activity of plant roots could explain this finding. Further studies are needed to reveal mechanisms controlling sertraline behavior in the soil-plant environment.

### **3.11.P-Mo221 Contamination of Water, Soil and Plants by Micropollutants from Treated Wastewater and Wastewater Treatment Plant Sludge**

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Several studies have shown that plants can absorb various micropollutants. One of the sources of micropollutants in the soil can be treated wastewater or sewage sludge. Previous studies were usually carried out in controlled conditions, i.e., greenhouses, etc. This study aimed to find out how the monitored compounds would behave in real conditions. For this purpose, nine raised beds were constructed at the wastewater treatment plants for České Budějovice, in which a mixture of vegetables (lettuce, onions, and carrots) or corn was grown in 2021. Several scenarios were considered: some beds were irrigated with treated wastewater, others were treated with sewage sludge, beds with composted sludge, and control beds (i.e., no source of contamination). Samples of treated wastewater and water drained from the beds, soil, and plants were taken during the year. Of the 77 substances analyzed, 59 were detected in wastewater and 11 in water that percolated through the beds irrigated with wastewater (e.g., telmisartan, gabapentin, and 1H-benzotriazole). Fourteen and ten compounds were found in sewage sludge and composted sludge, respectively, but only two substances (sertraline and 1H-benzotriazole) in water that leached through the beds with these sources of contamination. Compounds mostly did not accumulate in soils irrigated with wastewater. Low concentrations were found for telmisartan, carbamazepine, venlafaxine, and tramadol. In the case of both biosolids, gradually decreasing concentrations were observed for citalopram, sertraline, and telmisartan. Five substances were identified in lettuce and onion leaves and eight in carrot roots irrigated with wastewater (e.g., gabapentin, carbamazepine, and tramadol). In the case of corn plants, the compounds' concentrations were mainly below the limit of their quantification.

### **3.11.P-Mo222 Pharmaceuticals and Trace Metals Interaction Within The Water-soil-plant Continuum**

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The use of water resources highly impacted by the discharge of wastewater treatment plant (WWTP) effluents to irrigate crops may pose a global health risk related to food safety and soil quality. The objective of the study is to evaluate the effect of the interaction of pharmaceuticals (Phs) and trace metals (TMs) within the water-soil-plant continuum by considering plant uptake, the generation of transformation products (TPs), the dynamics in interstitial water, and the impact on the soil enzyme activity. We conducted a bioassay experiment with *Lactuca sativa* L. and an agricultural soil from an area irrigated with surface water impacted by WWTP effluents. Seedlings were grown during 45 days in a growth chamber and twice-a-week drip irrigated with synthetic water spiked with 7 TMs (100 µg L<sup>-1</sup>), 15 Phs (10 µg L<sup>-1</sup>), or 7 TMs + 15 Phs. A control (without spike) was included. The interstitial water was sampled weekly using rhizon samplers. TMs, major elements (MEs), Phs, and TPs were analyzed in leaves (internal and external), roots, soil, and water samples. Soil β-galactosidase, urease, phosphatase, and arylsulfatase activities were measured. Results show that the distribution pattern of some Phs, TMs, and MEs in plant tissues is significantly impacted by Ph-TM interaction. When Phs are added with TMs, diazepam tends to translocate towards the external leaves rather than remaining in the roots. On the contrary, the content of carbamazepine in external leaves is significantly higher when the irrigation water is not spiked with TMs. Metabolization processes are also observed. Several TPs are detected in different plant tissues, such as carbamazepine 10,11-epoxide, atenololic acid, o-desmethylvenlafaxine, paraxanthine, and cotinine. The TPs o-desmethylvenlafaxine and paraxanthine are not detected in the interstitial water suggesting their formation as a consequence of the plant metabolism. The Ph-TM mixture in the irrigation water has a significant negative effect on the uptake of TMs and causes a ME imbalance. Conversely, the levels of Cd in leaves are above the EU limits and detected levels are even higher in the presence of Phs. Concerning the interstitial water, Cr, Cu, and Zn concentrations are lower in the presence of Ph-TM suggesting that Phs favour TM sorption. Soil enzyme activity is stimulated in presence of Phs (with or without TMs), suggesting that the enzyme-producing microbial population is favoured.

### 3.11.P-Mo223 Understanding Bioavailability of Unregulated Organic Chemicals (UOCs) in Land Applications of Biosolids

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Biosolids (i.e., treated sewage sludge) are a highly underutilized resource for agricultural purposes. The land application of biosolids offers multiple benefits over the environmentally detrimental disposal methods such as landfilling and incineration. These benefits include improvements to soil health and plant nutrition. However, biosolids contain hundreds of unregulated organic chemicals (UOCs) leading to concerns that may hamper its expanded use on food crops and delay the realization of a circular economy. Therefore, a significant research need is to determine which biosolids-borne UOCs pose a high risk to ecosystem and human health when biosolids are land applied to agricultural crops at environmentally realistic rates. To begin addressing questions of environmental risks of UOCs, a plethora of data are necessary, including baseline occurrence, fate, and transport data, particularly for those UOCs that may be persistent in soil, chronically toxic, and/or bioaccumulative. Central to characterizing environmental risks of biosolids land applications is the mobility and bioavailability of biosolids-borne UOCs, which dictate the offsite transport potential and likelihood for bioaccumulation by food plants and/or soil-dwelling organisms such as earthworms.

In this study, we evaluated the use of thin-film passive samplers as a biomimetic tool to estimate the bioavailability of priority UOCs in biosolids-amended soil and assess the feasibility of using such bioavailability measurements to predict their bioaccumulation in earthworm and edible plant tissues. The thin-film passive samplers were calibrated and optimized through laboratory experiments. They were then imbedded in field plots amended with biosolids at different rates. Passive samplers, along with soil samples, earthworm samples, and various vegetables (at maturity) were collected and analysed for about 40 target UOCs. Our results are expected to allow for rapid assessment of chemical bioavailability and prediction of bioaccumulation for an extremely diverse chemical mixture and contribute to a holistic human health and ecological risk assessment. In this presentation, we will provide an overview of our research on the development of the thin-film based bioavailability method for UOCs, and applications under field conditions.

### 3.11.P-Mo224 The Biodegradation and Sorption of Pharmaceuticals in Amended Soil with Bio-based Fertilizers

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The application of bio-based fertilizers (BBFs) is a promising avenue to close nutrient loops and increase the sustainability of agriculture world-wide. However, the application of BBFs also holds the risk of introducing harmful organic pollutants to the soil. Numerous studies on pharmaceuticals focus on aquatic environments or sediment, whereas relatively little is known about their fate in soil.

Biodegradation and sorption are two fundamental processes that determine the persistence and bioavailability of organic pollutants like pharmaceuticals in the soil [1, 2]. Their sorption can be expected to be compounds specific, as it is related to specific functional groups and the biogeochemical characteristics of the soil. The introduction of pharmaceuticals to the soil via BBF application could influence their subsequent biodegradation and sorption behaviour. In the soil via interactions with the BBF matrix such as sorption prior to application.

To fill this knowledge gap, we employed soil biodegradation experiments to explore the fate of 9 common pharmaceutical compounds added directly and via BBFs in an agricultural field trials as part of the large, ongoing European research project

LEX4BIO. Subsequently, the half-lives and biodegradation rates were calculated to indicate their biodegradation and persistence. Finally, sorption experiments were employed to unravel underlying mechanisms governing the observed patterns.

### **3.11.P-Mo225 Environmental Fate of the Newly Discovered Sulfonated and OH-Sulfonated-PCBs: A Preliminary Evaluation**

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More than one hundred environmental contaminants belonging to the new classes of sulfonated and OH-sulfonated-PCBs were recently discovered. These chemicals originate from PCBs, but the metabolic pattern leading to their formation is not clear yet. One hypothesis is the oxidation of PCBs by Cytochrome P450 followed by the action of glutathione S-transferase (GST) and other enzymes.

Sulfonated-PCBs were first discovered in polar bear serum and then they were also measured, along with OH-sulfonated-PCBs, in the National Priority Site (SIN) for Remediation (SIN Brescia-Caffaro). This site, located in Italy, was heavily contaminated by the Caffaro plant which produced PCB mixtures until 1984 for various industrial applications. These new PCB metabolites, representing approximately 1% of parent compounds in soil, are not well characterized yet. Neither information on their precise chemical identity, their measured physical-chemical properties, their fate in the environment nor on their ecotoxicity and toxicity is available. Therefore, more attention should be focused on these new environmental contaminants to understand their potential environmental risk.

It has recently been demonstrated that sulfonated and OH-sulfonated-PCBs can bioaccumulate in earthworms and enter the food chain. They could also represent a risk for surface water and groundwater contamination, as their polar nature may enhance their movement with runoff and percolating water into the soil. Hence the importance of investigating the environmental fate of the novel PCB metabolites, which is the aim of the current work. To do so, different experiments were performed: adsorption to soil, degradation in soil (using different plant species selected based on their ability to degrade PCBs), bioaccumulation in roots and leaching behaviour in a soil column. The results obtained showed that sulfonated and OH-sulfonated-PCBs are persistent chemicals, might accumulate in biota and easily move to deeper soil layers due to their low soil-water partition coefficient (K<sub>d</sub>). These preliminary data represent the first experimental information regarding their behaviour in the environment and will be useful for further investigations.

### **3.11.P-Mo226 Back to the Roots: The Impact of Roots on the Uptake and Translocation of a Model Compound in Hydroponic Cultivated Tomato Plants**

**Alexander Dorn**, Tobias Breuer, Thomas Augustin, Klaus Hammel and Marc Lamshöft, Bayer AG - Crop Science Division, Germany

Plant uptake of chemicals by roots is an important component of the environmental risk assessment. In soil as well as in hydroponic solutions, the uptake of Carbendazim was retarded. It remained unclear, to what extent the physiology of roots regulated the uptake of Carbendazim. There are two different transport pathways in the roots, namely the apoplastic pathway via the cell walls and the symplastic pathway throughout the cells. The casparian strip is a barrier for the apoplastic pathway directing it to the symplastic pathway. Entering the symplast and finally the xylem requires the passage of a lipid membrane, which steers the translocation of nutrients or chemicals from the roots to the shoots.

In this work we aim to elucidate the uptake and subsequent translocation of Carbendazim using hydroponic studies with intact plants and such with their roots cut off, so that Carbendazim could enter the phloem and xylem directly. The differences observed between these experimental setups are analysed for a better mechanistic understanding of root uptake and translocation processes of PPP in plants. The hypothesis is that the Casparian stripe represents a barrier which slows down the transport of Carbendazim and retards the translocation to the above ground parts of the plant.

The study results will allow to answer the hypothesis that the uptake of Carbendazim is retarded by the root of tomato plants and to quantify the temporal development of Carbendazim concentrations in different plant compartments. Potential differences of transpiration and Carbendazim mass flow between the plants with and without roots will be evaluated and be discussed. This work is part of a project investigating uptake, transformation and translocation processes of chemicals in the soil plant continuum. The experimental results will be used also for simulation studies with multicompartment plant models in order to improve a mechanistic understanding of these plant processes. Details will be presented during the meeting.

### **3.11.P-Mo227 MALDI Mass Spectrometry Imaging to Track the Uptake and Systemicity of Agrichemicals in Crop Plants**

**Michael Kubicki**, Marc Lamshöft and Andreas Lagojda, Bayer AG - Crop Science Division, Germany

Agrochemicals such as fungicides, herbicides, or insecticides are widely used worldwide to control weeds, pests, and diseases to reduce the loss in crop production and increase yield and quality of crop products. Successful control and safe usage of these types of compounds depend on the compound reaching the target site within the crop plant. Therefore, monitoring agrochemical distribution within plant tissues delivers significant insights into agrochemicals adsorption, distribution, metabolism, and elimination (ADME). Visualizing and quantifying agrochemical uptake often requires a combination of techniques which usually utilize isotope labelling such as radioactive beta emitters like <sup>3</sup>H or <sup>14</sup>C and include combustion analysis, scintillation counting, autoradiography, and radio-high-performance liquid chromatography (HPLC). A major limitation of these methods is that they rely on radiosynthesis. High-resolution mass spectrometry combined with chromatographic techniques are used to assess ADME parameters by offering a powerful platform for the detection and characterization of plant protection products and their

metabolites. It provides accurate mass measurements by large full-scan sensitivity, leading to structure information of unknown biotransformation products. However, only limited information about the spatial distribution of target analytes is available due to the extraction step prior to analysis. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) has become a valuable analytical tool and can be used as a label-free, semi-quantitative alternative technique to visualize the spatial distribution of a range of compounds like agrochemicals and endogenous compounds (e.g., amino acids, polysaccharides, proteins) *in situ*.

Several mass spectrometry imaging experiments of selected plant tissues (e.g., roots, stem, leaf) were carried out on a Q-Exactive HF-X™ Hybrid-Quadrupole Orbitrap mass spectrometer, coupled to an AP-SMALDI5AF ion source. Mass spectra with high mass accuracy and high mass resolution were acquired for the precise identification of target molecules by the detection of monoisotopic masses of interest. Measurements were performed with a high spatial resolution of 20 – 50 µm step size to distinguish the distribution of selected target molecules in particular areas of interest. The obtained spatial information delivers new insights into the root uptake and systemicity of the agrochemicals within plants.

### 3.11.P-Mo229 OECD Round Robin Test to Evaluate a New Test Design to Determine Plant Root Uptake for Regulatory Environmental Fate Modelling

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In 2018 an OECD project lead by the German Federal Environmental Agency (UBA) started to develop a test guideline for the determination of plant root uptake for regulatory environmental fate modelling. The project builds on the study design that was developed from the outcome of a stakeholder workshop and subsequent discussions with academia, regulatory and industry. The OECD experts from academia, industry and regulatory who are working on the project recommended testing 14- and 28-days old plants instead of testing plant of uniform age. Additionally, uptake should be tested at three test item concentrations (0.5, 1.0 and 2.0 µmol/L) instead of one and the incubation period should be reduced from 8 days to of 48 h. Moreover, modifications to several experimental parameters were suggested.

After a pretest with wheat and oil seed rape plants had confirmed that the suggested changes were technically feasible, a round robin test (10 laboratories) was conducted in 2021-22. Uptake of [<sup>14</sup>C]-1,2,4-triazole in a hydroponic test system was studied at concentrations of 0.1, 1.0 and 10.0 µmol/L with 15- and 30-days old wheat plants that were incubated for 48 h at pH 5.5 and pH 7.5.

The average Transpiration Stream Concentration Factor (TSCF) value was  $0.54 \pm 0.19$  over all test concentrations and both pH values. The low data variance demonstrated the robustness of the test design. The results confirmed that significant biomass increase, and water consumption correlates with a consistent root uptake of the test item. Furthermore, a radioactive recovery between 95-105 % is an indication for a reliable test.

Additional important learnings from the test should be considered in the OECD test guideline, e.g., the relevance of accurate determination of evaporation from the hydroponic test system, describing plant growth with BBCH codes instead of plant age in days.

The overall results will be acknowledged to set up the test protocol of a new OECD guideline in 2023/24. The final version of the study design of the intended OECD guideline is considered appropriate to produce reliable data on plant uptake to be used as input for refined exposure modelling. An explicit guidance, on how to integrate the study design into the regulatory process is aimed to be developed based on the final OECD test guideline.

### 3.11.P-Mo230 Are Existing Lab-Based Equations Suitable to Predict Organic Contaminant Bioaccumulation in Plants in More Realistic Conditions?

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Organic contaminant (OC) accumulation in plants from air and soil represents the first step in the food web uptake, affecting both human and ecosystem exposure and effects. Therefore, the quantification and the prediction of OC plant uptake is fundamental for assessing ecological risk.

To estimate such uptake, several equations are available in the literature, mostly developed in lab conditions, to obtain the root and the stem concentration factors (RCF and SCF). These equations are used even in regulatory predictive approaches to predict the exposure through the food chain.

Many of the studies performed so far mainly included short-term laboratory and greenhouse experiments, using hydroponic systems or spiked soils. These types of experiments allow to produce results under controlled conditions, but they generally diverge from realistic field conditions. Plant uptake experiments performed in field-like conditions as well as field studies could provide important information to improve the ecological realism when modeling the uptake of OCs by plants.

The aim of this work was to assess the suitability of the existing lab based predictive equations in estimating the root and stem uptake of Polychlorinated Biphenyls (PCB) for several herbaceous and woody species. More specifically, we performed a long-term (18 months) greenhouse experiment with a weathered contaminated soil and five plant species and a field study in a heavily PCB contaminated agricultural site. More than 80 PCB congeners were measured in soil and plant biomass by GC-MS. The obtained data were used to develop new RCF and SCF predictive equations considering more realistic conditions that were later compared to those reported in the literature.

Concerning root uptake, the existing laboratory-based predictions were considerably lower, up to about 2 orders of magnitude (especially at increasing hydrophobicity) with respect to semi-field and field results. For stem uptake equations the difference was even higher (up to about 3 orders of magnitude) probably because of the relevance of another uptake path (i.e., air).

This study highlighted that the existing lab-based equations underestimate the bioaccumulation in plants; therefore, semi-field and field studies are necessary to obtain more realistic predictions of OC bioaccumulation.

### 3.11.P-Mo231 Do 17-B-Estradiol and Diclofenac, Found in Wastewater Reused for Irrigation, Have Genetic Effects on Crops?

**Andrea Lorena Garduno Jimenez<sup>1</sup>** and **Laura Carter<sup>2</sup>**, (1)University of Leeds, United Kingdom, (2)School of Geography, University of Leeds, United Kingdom

Contaminants of emerging concern (CECs) such as pharmaceuticals, antibiotics and natural and synthetic hormones, have been shown to be ubiquitous in the agricultural environment. This is mainly due to otherwise beneficial practices such as wastewater reuse for irrigation and replacing synthetic fertilisers with wastewater derived biosolids and animal manures and slurries. CECs have been shown to alter the gene expression of crops which can result in negative impacts on crop health; for example by causing flowering times to be out of synch with pollinator's lifecycles. There is an urgent need to understand the effects CECs on crops at environmentally realistic concentrations and application scenarios.

A greenhouse experimental trail to explore the fate and effects of two CECs of interest, namely the prevalent natural steroid hormone 17b-estradiol and non-steroidal anti-inflammatory drug diclofenac is near completion. To date, 17b-estradiol has been shown to promote plant growth, whilst diclofenac has been shown to hinder it, and an explanation for this effect remains elusive. A genetic mechanistic understanding of the effect of these two CECs will lead to a better understating of how these pollutants can affect crops at a genetic level. This study compares the gene expression (using proteomics), the uptake and metabolism (using targeted and untargeted liquid-chromatography mass spectrometry and metabolomics) of 17b-estradiol diclofenac on the different parts of the radish plant. The results will indicate whether current realistic exposure scenarios are of concern when reusing waste streams in agriculture. Furthermore, understating genetic mechanistic effects of two CECs known to have opposite effects will reveal how these pollutants can effect crops at a genetic level.

### 3.11.P-Mo232 Ecotoxicity Risk Assessment of the Plant Biostimulant Strigolactone (SL-6)

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A risk assessment on the aquatic toxicity of the plant biostimulant strigolactone (SL-6) was performed using a suite of standardised bioassays representing different trophic groups and acute and chronic endpoints. In freshwater, three trophic groups of algae, crustacea and fish were used. Whilst in marine waters, algae (unicellular and macroalgae), Crustacea and Mollusca were employed. In addition, the genotoxicity of SL-6 was determined with the comet assessment performed on unicellular algae, oysters and fish embryos. This is the first time ecotoxicity tests have been performed on SL-6, which is a phytohormone developed for agricultural use. In freshwater, the lowest LOEC was measured in the unicellular algae at 0.31 mg/L SL-6. Although, similar LOEC values were also found for malformations in zebrafish embryos (LOEC 0.33 mg/L). Consistent malformations of pericardial oedemas and yolk sac oedemas were identified. In marine species, the lowest LOEC was found for *Tisbe battagliai* mortality at an SL-6 concentration of 0.1 mg/L, this was also the lowest LOEC in all species tested. Significant genotoxicity was observed above control levels at 0.031 mg/L SL-6 in both unicellular algae and the zebrafish embryo. Genotoxicity was also observed in the oyster embryos but at higher concentrations with a LOEC of 0.03 mg/L. When applying the simple risk assessment, calculated predicted no effect concentration (PNEC) for the ecotoxicity tests and the genotoxicity tests were 0.3 µg/L and 0.01 µg/L respectively based on conservative assessment factors.

### 3.11.P-Mo233 Effects of BPA, BPS, and their Mixtures on Seed Germination – Assessed by the Combination of Germination Index (GI) and LC-MS Based Accumulation of Chemicals and Metabolites Profiling

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Substances of the bisphenol group are well-known environmental endocrine disruptors and their adverse effects on different environmental organisms and even humans have been assessed by many different methods. Wide spread use of these substances increases the detected areas and concentrations of them and this may lead to the potential risks of the environment and humans. Among all these substances, bisphenol-A (BPA) and bisphenol-S (BPS) are of special concern due to their high volume of usage compared to the other substances. Therefore, analysis of the effects from the existence of BPA and/or BPS in soils with environmentally relevant concentrations is of interest. Phytotoxicity microbiotest has been used to monitor environmental pollutants in soils and sediments, and the effects by toxicants can be estimated by germination index (GI) calculated from the seed germination and root elongation. In this work, different concentrations of BPA, BPS, and the mixture of BPA and BPS were exposed to *Sinapis alba* (*S. alba*) seeds and the effects were analyzed by GI after 3 days of exposure. In addition, accumulation of BPA and BPS to each of three different compartments, roots, stems, and leaves, were quantified by HPLC-MS analysis. GI for BPA and BPS exposure of 1, 10 and 25 µM are calculated as  $124.9 \pm 5.2$ ,  $134.0 \pm 19.0$ ,  $98.6 \pm 12.1$  and  $145.4 \pm 18.8$ ,  $105.9 \pm 28.0$ ,  $68.2 \pm 9.4$ , respectively. These results indicate that the seeds exposed to higher concentration of both BPA and BPS may have some inhibition effects but exposed to lower and environmentally relevant concentrations of both BPA and BPS may have some promotion effects. Accumulation of the substances on each compartment was different for the exposed substances, having accumulated BPA and BPS concentrations on roots, stems, and leaves for 25 µM exposure were 0.051, 0.020, and 0.026 µM, and 0.520, 0.033, and 0.282 µM, respectively. Water solubility may have a big role on the accumulation results due to about 10 times higher water solubility for BPS compared to BPA. Using high resolution LC-QToF-MS, DIA non-targeted metabolomics was



conducted. For this data analysis, MS-Dial, a powerful open science tool for MS annotation, was used and meaningful data were achieved. This combination method of GI, quantitative analysis, and *in Silico* annotation methods for metabolites would provide the future directions regarding higher plant phytotoxicity bioassay due to the deliverable and detailed data on the effects.

### 3.11.P-Mo234 Pharmaceuticals and Antibiotic Resistant Genes Detection in Horticultural Soils After Irrigation With Reclaimed Wastewater

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The demand for water suitable for irrigation in areas with freshwater scarcity implies adequate wastewater treatment systems that do not generate a risk for agroecosystems, including the organic micropollutants commonly found in wastewater (e.g., pharmaceuticals). Under the H2020 HYDROUSA project a wastewater treatment system has been installed in the Greek island of Lesbos, consisting of a combination of upflow anaerobic sludge blanket reactor, a constructed wetland, and UV disinfection. The objective of this study was to evaluate the input of pharmaceuticals, from the reclaimed water (RW) into arable soils, during the growth cycle of lettuce crops during summer and winter. Tap water (TW) was used as a negative control to irrigate the soil. Soil samples (NS) were taken before the first crops irrigation, two weeks after, and at harvesting time (40 days). Once the plant was harvested, the bulk soil (BS) attached to the roots was collected individually. The roots were then soaked in a NaCl solution (RS), and the soil bonded to the roots interface was collected in the solution, which finally was filtered to separate the solids (for microbiological analysis). For the pharmaceutical analysis, ultrasound extraction and SPE were performed for the NS and BS soils. The RS solutions were extracted by SPE. A total of 70 compounds were analyzed in a quadrupole linear ion trap tandem mass spectrometry (UHPLC-QqLIT). Pharmaceuticals (more than 15 analytes) were detected in soils at both campaigns, and in the different types of samples (soils and rhizosphere solution). A higher occurrence and concentration of compounds was observed in the BS when compared to NS, in the plots irrigated RW but not for the TW. Analgesics and antiinflammatories were the most detected group ( $p < 0.05$ ) in both seasons and in every irrigated soil. Summer campaign showed higher concentration for most of the compounds, especially for cardiovascular and psychiatric drugs ( $p < 0.05$ ), which were found in a range (sum) between 0.036-236  $\mu\text{g/kg}$  and 0.055-302  $\mu\text{g/kg}$ . While antibiotics were less frequently detected than other pharmaceuticals, possibly due to their shorter half-lives in soils, detections of sulfamethoxazole and enrofloxacin indicate their reach to soils. Additional microbial analysis for the determination of antimicrobial resistant genes are being processed at the moment, and will contribute with information regarding the impact of the influx of these pollutants on the soils.

### 3.12 HR-MS Analysis of Metabolites and Transformation Products of Organic Pollutants - What Remains Unknown

#### 3.12.P-Th202 In-Stream Attenuation of Pharmaceuticals and Their Transformation Products in Intermittent Streams

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The increasing use of pharmaceuticals and hence their widespread occurrence in the aquatic system arises concern on their potential ecological and human health effects. But what happens once in the environment? In the environment, pharmaceuticals may suffer from different stressing conditions due to solar irradiation, temperature, microorganisms, undergoing transformations by means of hydrolysis, photolysis or biodegradation forming transformation products (TPs). Little is known about their fate and possible natural in-stream attenuation by either biodegradation or photolysis, thus to expand our knowledge on the occurrence of pharmaceuticals and their TPs, both laboratory and field studies were performed. Two intermittent rivers were studied from Spain and Greece where the discharge effluent as well as the upstream and downstreams points were analysed. Samples were extracted using solid phase extraction and analysed using liquid chromatography coupled to a high resolution mass spectrometer (LC-HRMS). Target analysis of over 100 pharmaceuticals was performed and over 50 pharmaceuticals were detected and quantified. At the same time, lab-scale photodegradation studies were performed to identify and detect transformation products. Venlafaxine and losartan were frequently detected and the generation and identification of the corresponding photo-transformation products (photo-TPs) under lab-scale photolysis was studied. A retrospective analysis was then performed to search for these photo-TPs in the real samples. Concerning venlafaxine four TPs were detected however it remains very challenging to be able to distinguish between human metabolites and natural in-stream attenuation (biodegradation or photolysis). Hence further studies are necessary to better understand the fate of these contaminants and to be able to assess the health of a watercourse.

#### 3.12.P-Th203 Biological Transformation of the Insect Repellent Icaridin and its main Transformation Product Icaridin-acid – Kinetics and Transformation Pathway

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Icaridin (ICN) is a widely used insect repellent and a substitute for *N,N*-Diethyl-*m*-toluamid (DEET) since the end of the 1990's. ICN is removed rather efficiently in waste water treatment plants (WWTPs) likely by biological transformation, which might explain, that it has been rarely detected in the aquatic environment. However, already back in 2004 T. P. Knepper showed, that ICN is mainly transformed to icaridin-acid (ICN-acid) by oxidation of the primary alcohol of ICN. The further transformation of ICN-acid was not elucidated in this study and due to lacking commercially available reference standards, knowledge about the occurrence and environmental fate of ICN-acid is still scarce. To fill this gap, a reference standard of ICN-acid was synthesized

and characterized in-house. To investigate the degradation kinetics and further transformation of ICN-acid, batch experiments were performed in a mixture of activated sludge and effluent taken from the WWTP Koblenz (Germany). For the reason of comparison, experiments were also conducted with ICN. Both, ICN and ICN-acid, were spiked in various concentrations between 10 and 1000 µg/L and incubated under oxic conditions. Furthermore, one non-spiked control and one autoclaved control with a spike level of 100 µg/L (ICN and ICN-acid) were used to facilitate identification of transformation products (TPs) and differentiate between abiotic and biotic degradation, respectively. Samples were taken repeatedly during an incubation period of 21 days and analyzed by triple quadrupole mass spectrometry for quantification and determination of degradation kinetics. First results confirm that ICN is mainly transformed to ICN-acid and that this TP is further transformed by biologically-mediated processes. The degradation kinetic of ICN was faster ( $k_{\text{biol}} = 31 \pm 2 \text{ L g}_{\text{ss}}^{-1} \text{ day}^{-1}$ ) than that of ICN-acid ( $k_{\text{biol}} = 2 \pm 0.1 \text{ L g}_{\text{ss}}^{-1} \text{ day}^{-1}$ ). For detection and identification of the TPs of ICN-acid, measurements via high-resolution mass spectrometry will be performed. Degradation kinetics of ICN and ICN-acid and proposed transformation pathways of ICN-acid will be presented at the conference. Furthermore, environmental samples will be analyzed afterwards to detect ICN-acid and the identified TPs in the aquatic environment. With the results of this study, the degradation behavior and occurrence of ICN and ICN-acid in the environment should be unraveled and thus also the potential impact on the environment assessed in more detailed.

### 3.12.P-Th204 Analysis of Urinary Biomarkers for the Selection of the Most Suitable Personal Protection Equipment for E-waste Dismantlers

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Recycling electronic waste (e-waste), which includes electrical goods such as computers, TVs, and mobile phones, has gained importance. This is primarily because these gadgets are constructed mostly of precious and reusable materials like plastic and metal. Recycling e-waste facilities might be regarded as hot spots of air pollution, considering how much particulate matter (PM) is released during working hours. These emitted PMs contain hazardous materials like plasticizers and flame retardants (FRs), which are proven to have toxic effects on human health. Previous research [1] has described how plastic additives are present in the air of recycling plants and how their employees have been exposed to them. As a continuation of this study, the main objective of this work is to study the use of precautionary measures, in particular the use of personal protective equipment (PPE), to reduce the e-waste dismantlers' exposure to these contaminants. Two different facemasks (Surgical and FFP3) were compared. The assessment was done by evaluating the exposure through the analysis of metabolites in urine.

For that purpose, an intensive sampling campaign has been performed in two working areas of a recycling facility located in Catalonia (an area for cathodic ray-tube (CRT) TV dismantling and a grinding area). A group of selected volunteers wears, during all their working days (8 hours) one of the compared facemasks. Then, the first urine sample of the following morning was collected for analysis. Additionally, the workers also wore silicone necklaces during their work day. They were used to assess human exposure to these hazardous substances associated with plastic. Analysis was performed using LC-MS/MS methodologies, and different plasticizers and FRs were determined, including organophosphate esters (OPEs), and phthalates, among others.

High levels of plastic additives are found in silicone necklaces, which demonstrates that e-waste dismantlers are exposed to these contaminants by inhalation and dermic absorption. Additionally, urine samples confirm the exposure suffered, since the biomarkers of these chemicals are present. Although the exposures suffered by the workers in the same area are similar, the urine samples show differences between the workers that wear FFP3 and surgical masks. Workers that wear FFP3 masks present lower levels of biomarkers, which gives evidence of the higher protection presented by these facemasks against plastic additives.

### 3.12.T-01 Molecular Insights into Biodegradability of Ozonation Products from Effluent Organic Matter

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Ozonation has so far been assumed to increase the biodegradability of effluent organic matter (EfOM), but some compounds have been found to produce stable ozonation products (OPs) resistant to biological degradation. Recent advancements in analytical methods, specifically isotopic labeling with heavy ozone and analysis of resulting EfOM OPs by liquid chromatography coupled to ultra-high resolution mass spectrometry, have broadened our understanding of ozonated EfOM composition. So far no studies have investigated on a molecular formula level the biodegradability of OPs from EfOM.

Wastewater effluent was ozonated with mass labeled (<sup>18</sup>O) ozone to generate labeled OPs. The effluent was inoculated with biologically active preconditioned sand and underwent biodegradation for 28 days. The OPs were detected with LC-FT-ICR-MS. While most molecular formulas detected in ozonated EfOM are biodegradable, just over a quarter were recalcitrant to biodegradation. Most CHNO and CHNOS formulas are readily biodegradable, but 28% and 16%, respectively, are recalcitrant. Of the labeled OPs, 31% remain detectable after 28 days. The majority of recalcitrant labeled OPs are from the CHO and CHNO formula class. Some of the CHNO formulas may have N-oxide functional groups, a stable functional group formed during ozonation.

While most products with <sup>18</sup>O have similar rates of removal among the isotopologues in a series, some <sup>16</sup>O isotopologues are recalcitrant with their <sup>18</sup>O counterparts readily biodegradable. The difference in detected biodegradability implies the possible formation of OPs during biological treatment.

Track: 3. Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modelling

### 3.12.T-02 An Interactive App to Curate Transformation Product Information from Text Mining Results

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An important consideration for understanding the hazards posed from different compounds is the transformation products (TPs) they form once they enter the environment. Unfortunately, there is much less information available for TPs than their parent compounds, while most of the information is text based in journal articles and databases. This makes it difficult to access and include into identification workflows based on high resolution mass spectrometry (HR-MS) and other analyses. One such database with text-based TP information is PubChem, which contains a lot of TP information, especially in the "Metabolism and Metabolites" section. In addition, PubChem runs a text mining software (LeadMine), which identifies chemical names in the text-based sections of the database. To better utilize this information, we have built a shiny app which will allow users to go through the text mined information in the Hazardous substance databank (HSDB) dataset to identify which of the extracted chemical names are TPs. After downloading the entire HSDB dataset and filtering based on the precursor compounds of interest, the app will display the structure of the precursor and the potential TP extracted via text mining, together with the HSDB text snippets to allow the user to identify whether the extracted structure is indeed a TP of the precursor. The information can then be saved in a table and downloaded for further analysis, or central upload as formal transformation reactions. In addition, the user has the option of saving the chemical names of TPs not identified by the text mining software together with their SMILES, to fill database gaps.

The app has so far been applied to compounds from several datasets (28 from the NORMAN-SLE and 8 from the PubChem PFAS Tree, as well as 33 PBT compounds on the SIN-list). Of the 366 chemicals identified by the text mining software, 60 were new reactions that were not already available in the transformation products dataset in PubChem, the majority of which were found among the NORMAN and PBT compounds. Among these reactions were transformation products of azo dyes such as benzidine which has carcinogenic properties. In addition, some persistent substructures were identified in the TPs. This shows promise for expanding the current TP datasets available in databases such as PubChem as well as for improving TP suspect lists and furthering the understanding of the fate of environmental contaminants.

### 3.12.T-03 HRMS Analysis of Antimicrobial Peptide Biotransformation Mediated by Extracellular Wastewater Peptidases

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Substantial fractions of human-administered antibiotics are secreted into wastewater streams in their still active form. When antibiotics are not removed during the wastewater treatment process, they can enter the environment and have adverse effects on ecosystems. It is assumed that the presence of antibiotics in wastewater as well as in natural systems contributes to the emergence and spread of antibiotic resistance genes in these systems. Several antimicrobial peptides (AMPs) emerged in the last decades as particularly promising antibiotic candidates because of their low tendency to evoke resistance in pathogenic bacteria. What remains unknown is whether and how AMPs are transformed during the wastewater treatment process and whether transformation would result in an inactivation of the AMPs. To shine light on the fate of AMPs in wastewater, we quantified extracellular peptidase activities in wastewater at different stages of the treatment process at four different full-scale WWTPs. Finding highest extracellular peptidase activity in raw wastewater, we incubated a set of ten different AMPs with cell-free extracts of raw wastewater. We quantified AMP concentrations during these incubations using liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS) and found large variations in the susceptibilities of the different AMPs to hydrolysis by wastewater peptidases. For rapidly hydrolyzed AMPs, we performed suspect screening to identify products of single amide hydrolysis events. We subsequently selected the most persistent hydrolysis products and obtained custom-synthesized peptide standards for an absolute quantification of product concentrations. By comparing the transformation products for all WWTP extracts, we found a high similarity of formed products between the WWTPs, showing that peptidases in raw wastewater are not only specific, but seem to have some conserved specificity across the tested WWTP influents. Overall, this study provided insights into peptidase specificities of raw wastewater and the findings might contribute to the development of sustainable peptide-based antibiotics.

### 3.12.T-04 What Sample Information Is Lost from Sampling to Analysis During Non-Target Analysis?

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Non-target analysis is becoming a popular tool for elucidation of chemical constituents, including chemicals of emerging concern and their transformation products, and comparison of samples, without the need for prior knowledge. However, reliability and reproducibility of obtained results can be influenced by changes in a sample over short or long term (i.e. within timeframes of analysis). For example, for some compounds, degradation starts immediately following sample collection, and is then influenced by temperatures and time until analysis. So far, most research has looked into degradation of specific compounds, via target analysis, so that results can later be corrected using chemical-specific degradation rates. While this is not a viable option for non-target analysis, due to the number of analytes, knowledge of the fraction of sample loss between sample collection and analysis is important for the understanding of chemical fate and risk. Therefore, we aimed to document the change in sample profile in the immediate-to-short term i.e. the first 24 hours following a typical wastewater sampling scenario, using a non-target approach. For this we obtained a wastewater sample from a manhole in a suburban area in Australia and commenced measurement using Liquid chromatography coupled to High resolution mass spectrometry (LC-HRMS) within 90 minutes of sample collection. The sample

was then kept in the autosampler and re-analyzed 47 times over a 24-hour period and in triplicates each day over 6 days. Additionally, a sample kept at ambient temperature was measured on days 2 to 6 for comparison. Fold changes were calculated for the features in the aligned feature list, based on which features got classified as “Eliminated”, “Decreased”, “Unchanged”, “Increased” and “Formed”. Results show the decrease in intensity and elimination of some compounds over time, while others increase in intensity or have been formed as part of the transformation process. These findings will have implications for the use of NTA, especially when conducting temporal / spatial assessments.

### 3.12.P HR-MS Analysis of Metabolites and Transformation Products of Organic Pollutants - What Remains Unknown

#### 3.12.P-Th205 Multiple Deuterium Labelling: New Insights Into the Fate of Organic Pollutants in Natural Waters

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Organic pollutants (OPs) can undergo many interactions with molecules of dissolved organic matter (DOM) in water, e.g., to form bound residues (BRs) under solar irradiation. High resolution mass spectrometry (HRMS) is a powerful tool for the screening of complex reaction products from OPs, including BRs. While <sup>15</sup>N and <sup>18</sup>O have been used for the identification of BRs and transformation products (TPs) from DOM, generic stable isotope labelling is still missing for OPs with different elemental compositions. Deuterium (<sup>2</sup>H, or D) is a promising option due to its low natural abundance and universal binding to the carbon backbone. Using <sup>2</sup>H labelled chemicals during transformations lead to the formation of labelled TPs and BRs, but also results in an increase of false-positive assignments which cannot be easily validated by existing algorithms. Here, we used multiple deuterium labelling (DxL) to examine the formation of <sup>2</sup>H labelled TPs and BRs, and developed a data processing pipeline for the validation of <sup>2</sup>H formulas.

Data from photo irradiation experiments of carbamazepine (CBZ), an antiepileptic drug, were used for testing the robustness of the data processing pipeline. Irradiation experiments were conducted using a solar simulator. Duplicates of 50 µM CBZ and CBZ-D<sub>10</sub> were mixed with a DOM standard and irradiated for 24 h. Samples were analysed using a reversed-phase liquid chromatography system coupled to a Fourier-transform ion cyclotron resonance mass spectrometer.

Using a new data processing pipeline, false-positive molecular formulas could be identified and excluded, resulting in less than 1% multiple assignments. Over 4000 BRs were identified based on <sup>2</sup>H formulas. The 3-quinolinecarboxylic acid (3-QCA) transformation pathway, which has been previously described using <sup>15</sup>N labelling, was confirmed with corresponding D<sub>5</sub> formulas, indicating the robustness of the applied filters. However, D<sub>4</sub> formulas represented > 25% of all BRs, pointing to a previously unconsidered pathway of CBZ photolysis/BR formation. Overall, our results suggest that DxL in combination with HRMS and advanced data processing is a promising approach to investigate the formation of BRs and to study the fate of OPs in the environment.

#### 3.12.P-Th206 Phototransformation of Pharmaceuticals in Temporary Rivers Under Simulated Sunlight in Real Environmental Conditions

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Phototransformation of pharmaceuticals has mostly been investigated in permanent rivers and scarcely in temporary effluents, where the lack of dilution capacity led to an overall increase of pharmaceuticals concentrations. The major factors contributing to in-stream pharmaceuticals attenuation include sorption, photo- and biotransformations, but the relevance of each process and the transformation products (TPs) generated are compound specific. In the case of polar compounds which tend to remain in the water phase, photodegradation may predominate. However, the majority of phototransformation studies at lab scale do not include real environmental conditions, which should be covered for a better understanding of the attenuation mechanisms in water systems. In this work, we hypothesized that Cu might play a contribution in the photodegradation because several pharmaceutical classes are known to form photoreactive complexes with Cu. The objectives of this study were to investigate the presence of pharmaceuticals along two river stretches and perform phototransformation experiments to study kinetics, TPs, and reaction pathways of selected pharmaceuticals under simulated sunlight irradiation in the presence or absence of dissolved Cu<sup>2+</sup> / Fe<sup>3+</sup> ions in real environmental conditions. All samples derived by photolysis experiments were filtered and directly analyzed by liquid chromatography high-resolution mass spectrometry (LC-HRMS). The results showed the occurrence of 13 compounds in POCIS extracts, including β-blockers and psychiatric drugs, with most of them undergoing dissipation to different extent along the river stretches. Oxazepam, venlafaxine and fluconazole were selected for photodegradation experiments. In all cases, photodegradation resulted to be faster in the presence of Cu<sup>2+</sup> and Fe<sup>3+</sup> ions than direct photolysis, with the complete attenuation of oxazepam and venlafaxine after 21 hours experiment, and 80% attenuation of fluconazole after 20 hours. A total of 14 TPs were identified and confirmed. In most of the cases, the degradation pathways included oxidation and hydroxylation mechanisms with formations of TPs already found in previous studies; however, new TPs were identified for the first time in this study, especially TPs formed only in the presence of dissolved Cu<sup>2+</sup> and Fe<sup>3+</sup> ions. Some of the identified TPs were confirmed to be present in the real water samples, confirming the role of phototransformation in pharmaceuticals attenuation.

#### 3.12.P-Th207 Exploring the Profile of Pesticides and Transformation Products in Stormwater Events of Agricultural Area Using Extensive LC-HRMS Screening

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The intensive use of pesticides and the formation of active transformation products (TPs) are of increasing concern as they are intensively detected in agricultural areas. Stormwater event would transfer massive amount of pesticides to adjunct surface

waters, posing potential risk to aquatic organisms. Yet, comprehensive understanding for occurrences and profiles of pesticide and TP in stormwaters remains limited. In this study, the extensive screening method using LC-HRMS was developed not only for 384 targets, but also for suspects and unexpected substances. To enrich stormwater samples, the SPE method using a multi-layer cartridge was applied. High frequency composite sapling (sampling interval: 1hr) was conducted to explore time-dependent contaminant profiles during the stormwater events in an agricultural area. The target screening successfully measured 56 pesticides and 4 TPs in the stormwaters. The priority pesticides included bromobutide with the highest event mean concentration (EMC) of 1,460 ng/L, followed by bentazone (1,180 ng/L), bensulfuron-methyl (790 ng/L), and climbazole (670 ng/L), which are highly relevant to major crop in the study area. Neonicotinoid insecticides (i.e., imidacloprid, thiamethoxam, clothianidin) showed high risk quotient exceedance rates (>80%). Compound profiles were featured with maximum concentrations within 2-4 hours of initial rainfall (first-flush). The suspect and non-target screening (SNTS) was applied to identify TPs of pesticides. The non-target peaks were obtained with software-aid procedure (enviMass v4.1), and then were matched with exact mass of predicted TPs using feasible biological reactions for the degradation of pesticides. Further structure elucidation step for TPs was performed with the interpretation of data-dependent MS2 (ddMS2). Consequently, 32 TPs for 11 pesticides were tentatively identified with confidence level above 2. Typically, neonicotinoid insecticide showed analogous degradation products (nitroso→desnitro→urea). Pesticides and TPs showed rapid concentration increases within a week after the field applications, and then returned back to a base level after about 90 days. The occurrence profile of pesticides and TPs in agricultural field was highly affected by the application period and the precipitation intensity. The high RQ evaluation for neonicotinoids indicates the potential hazard to aquatic organisms in adjunct streams after stormwater events.

### 3.12.P-Th208 Simple and Rapid Quantification of Per- And Perfluoroalkyl (Pfas) Compounds in Sea Water

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The ocean has been termed by many a “terminal sink” for PFAS; with reports of PFOS, PFHxA and PFOA being abundant in surface and subsurface seawater. Seafood consumption is one major pathway for human PFAS exposure, however, the distribution and abundance of PFAS in seawater is still poorly understood. Recent advancements suggest that one source of PFAS in seawater could be due to petroleum being released into the sea.

The European Parliament and Council of the European Union have recently published directive that has set limits on PFAS in drinking water to 500 ng/L for all PFAS compounds identified, and 100 ng/L for a subset of PFAS compounds that are potentially detrimental to human health.

With PFAS regulations tightening and concerns of PFAS exposure from seafood, it is paramount we monitor the PFAS levels in seawater. We provide a robust and sensitive method for PFAS detection and quantification in seawater.

Mixed standards were prepared at a ratio of 2.5 mL LC-MS water to 2 mL 50:50 acetonitrile/methanol +0.22% formic acid to construct a calibration curve between 0.2-1000 ng/L. Samples were prepared with 2.5 mL of seawater from the Irish Sea added to 2 mL of 50:50 acetonitrile/methanol + 0.22% formic acid solution prior to analysis.

Chromatographic separation was performed using a Phenomenex Luna Omega PS C18, 100 Å, 100 x 2.1 mm, 3 µm, and a Phenomenex Gemini C18, 110 Å, 100 x 2.0 mm, 3 µm delay column. The injection volume was 50 µL. Mobile phase A was 20 mM ammonium acetate in water and mobile phase B was methanol. The MS was operated in electrospray ionization in negative ion mode.

The method is presented to quantify PFAS compounds in seawater down to the sub-ng/L range. Both an external standard calibration curve and standard addition workflows were implemented. The analytical method provides a simple, reproducible, and robust sample preparation with no SPE needed. Good chromatographic peak-to-peak separation was achieved with a total run time of 12 minutes. Excellent sensitivity was achieved with LOQ values at sub-ng/L levels. Accuracy (%) and average accuracy (%) were within acceptable criteria (70%-30%) and area %CV was ≤4.26% for spiked seawater samples against an external standard calibration curve, confirming robustness and reproducibility. An external calibration curve in solvent and a standard addition workflow was implemented to further confirm the concentration of PFAS compounds detected in un-spiked seawater.

### 3.12.P-Th209 Diurnal Profiles of Organic Marker Compounds in Atmospheric Secondary Organic Aerosol During Summertime in Melpitz, Germany

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Atmospheric aerosol particles consist of numerous organic substances which can have primary or secondary sources. Secondary organic aerosol (SOA) is formed by oxidation of biogenic and anthropogenic volatile organic compounds (VOCs) and can contain tens of thousands of different organic substances. The organic composition is not only highly complex but also subject to constant dynamic changes. Previous measurement campaigns with online aerosol mass spectrometers (AMS) provided a wealth of insights into bulk SOA, but are limited in terms of describing SOA at the molecular level. Time resolved measurements of individual molecules, which can serve as marker compounds of specific emission sources or formation pathways, are scarce. Therefore, in this study, particulate matter (PM) samples were collected at 4-hour intervals over six weeks at a rural site in Melpitz, Germany during the ACD@SummerHome 2021 campaign. The collected samples were subsequently analyzed by LC/HR-MS and IC/HR-MS to quantify 46 different organic compounds including mono-, di-, tricarboxylic acids, markers for monoterpenes, organosulfates, and inorganic anions. For example, the concentration of oxalic acid varied between 39 and 832 ng m<sup>-3</sup>, with higher values during the day than at night. Such a recurring diurnal profile can be observed for almost all investigated substances. Based on this, we examined the daily profiles within analyte groups. For the substance class of dicarboxylic acids, we noticed that the shorter the chain length, the later in the day the concentration maximum occurs. From the literature it is known that short-

chain dicarboxylic acids can be formed from long-chain dicarboxylic acids through photochemical processes. Our results are consistent with this, as the last oxidation product of dicarboxylic acids, oxalic acid, has its daily maximum between 2 and 6 pm. We found the same correlation for the oxidation markers of monoterpenes. The oxidation products of the first generation, pinic acid and pinonic acid, reach their maximum between 6 and 10 am, while late transformation products such as 3-methyl-1,2,3-butanetricarboxylic acid and diaterypenylic acid acetate peak in the afternoon and then drop again in the evening. Overall, the presented measurements help to understand atmospheric transformation processes both at the molecular level as well as at rather short time scales.

### **3.12.P-Th210 Non-targeted Analysis for Metabolites of ATBC and ATEC by LC-Q-ToF-MS after Exposure to Human Liver Microsomes**

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DEHP is a plasticizer that softens plastics and is widely used daily, but it is an endocrine disruptor and has a harmful effect on the human body. To replace DEHP, the use of alternative plasticizers is increasing. However, there is not enough research on the metabolic process of alternative plasticizers. This study was conducted using human liver microsome (HLM) to understand the metabolic process of alternative plasticizers through non-targeted analysis.

ATBC and ATEC were selected as target substances among alternative plasticizer substances judged to require metabolite search. Non-targeted analysis was performed using LC-Q-ToF-MS (ABSCIEX 7600) to identify the metabolites of ATBC and ATEC after exposure these substances to HLM.

As a result of non-targeted screening of metabolites of ATBC and ATEC using HLM, we were able to identify several metabolites which were known as metabolites of ATBC and ATEC in human experiments. In the case of ATBC, 9 metabolites were identified including TBC, ADBC, DBC and 10 metabolites were identified for ATEC including TEC, ADEC, DEC. We identified the metabolites of ATBC and ATEC through this study, and in the future, we plan to determine the biomarker of these alternative plasticizers and will also use these biomarkers for human biomonitoring.

### **3.12.P-Th211 Deconjugation of Diclofenac Glucuronide in Wastewater – Not As Straight Forward As We Thought**

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Diclofenac is a prominent example for the detrimental effects that human pharmaceuticals can have on ecosystems. After oral intake, diclofenac (DCF) is extensively metabolized and excreted mainly as conjugate metabolites. It has been demonstrated that deconjugation of glucuronide conjugates can lead to re-transformation of the parent compound. This process has not yet been tested empirically for DCF in wastewater but it offers an explanation for observed negative removal efficiencies of DCF during wastewater treatment. The aim of this study was to investigate the fate of DCF and three of its human metabolites in wastewater and activated sludge. Particularly, we aimed at studying the kinetics of the re-transformation of diclofenac glucuronide (DCF-gluc) to DCF under biotic and abiotic conditions. Laboratory batch experiments using raw wastewater and activated sludge were conducted in triplicate to assess to what extent deconjugation of DCF-gluc could play a role in sewer systems. We aimed at quantifying the kinetics of deconjugation of DCF-gluc in wastewater and activated sludge from secondary wastewater treatment. The concentration of DCF and its metabolites were measured via LC-MS/MS. Our preliminary results suggest that DCF-gluc disappears quickly (<1h) from wastewater not necessarily due to enzymatic deconjugation but due to pH-induced hydrolysis occurring from pH6 and above. This suggests that deconjugation of DCF-gluc cannot explain negative removal efficiencies observed at WWTPs because DCF-gluc might already be fully converted to DCF in the sewer system. Mass balances for each of the incubations indicated an increase of DCF of up to 134% over 24h. Relative to  $t_0$ , DCF concentrations increased most in the incubations containing activated sludge (+25%, 0.01 sd), while concentration in incubations containing only wastewater increased by 18% (sd 0.01) and 17% (sd 0.02). No significant increase was observed for the autoclaved control incubations (+2%, sd 0.01). Unexplained surplus concentrations of DCF between 5.8 and 18.9 µg/L were observed after 24 h. This suggests an additional yet unidentified source of DCF was present, particularly in the biologically active incubations.

### **3.12.P-Th212 Metabolite Screening of the Mycotoxins Enniatin B and Beauvericin**

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Plant products are increasingly being used as feed ingredients, and with this change comes new pollutant concerns. With this shift the contaminants of concern are changing from persistent organic pollutants to those associated with crop production and storage, like pesticides and mycotoxins. Mycotoxin contaminations arise from mold growth, and occur under interactions of complex environmental conditions that are shifting under climate change. Two emerging lipophilic mycotoxins, enniatin B (ENN) and beauvericin (BEA), were recently reported in aquafeeds and farmed fish, but are not currently regulated. Both mycotoxins have demonstrated toxicity and are metabolized in mammalian and avian species, but knowledge on metabolism in fish remains limited. In this study, a novel non-targeted screening workflow was applied to unravel the metabolites of the two mycotoxins. Samples were obtained from a 3-month controlled feeding trial in which Atlantic salmon were fed a control diet, or diets spiked with ENN or BEA. Tissue samples (liver, kidney, muscle, and brain, 0.4-0.6 g) were extracted and screened on two ion mobility(IMS)-HRMS instruments against a library that included *in silico* predicted candidates along with predicted retention time (RT) and collision cross section (CCS) values. Data were processed after peak picking by applying the filters for mass error,

theoretical fragments, RT, and CCS. Data were examined graphically to select clusters of tentative detections that had unique RT and CCS profiles in treated samples. We confirmed ENN in 12 of 12 samples, and the internal standard in 30 of 36 samples. BEA was observed in only 3 of 9 samples. Most of these “false negatives” detections are likely due to higher limits of detection on the QToF instrument compared to QQQ. RT and CCS predictions were within 1.2 minutes and 2%, respectively for the mycotoxins. We found two types of metabolites for ENN: hydroxy- and dihydroxy-ENN isomers, chiefly in liver samples Hydroxy- and dihydroxy-ENN had matches with 5 and 2 theoretical fragments, respectively. Other metabolites were not detected, namely demethylated variants, which have been reported in *in vitro* studies. No metabolites of BEA were detected. The non-target screening strategy applied demonstrates how IMS-HRMS and prediction tools can provide new knowledge towards a group of contaminants of emerging concern under climate change, in a more time- and cost-effective manner.

### 3.12.P-Th213 Measurement and In Silico Prediction of Pharmaceutical Biotransformation in Receiving Water

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Water and effluent quality monitoring methods in the UK are predominantly focused on organic matter (nitrogen, phosphorus levels) whereas pharmaceuticals and their transformation products (TPs) are not monitored routinely in river water. A European Commission report in 2020 details a shortlist of several anti-microbial pharmaceuticals in the next EU Water Framework Directive (WFD) “watch list” to be closely monitored in inland surface waters and to further the understanding of any effects. Antimicrobials such as sulfamethoxazole and clotrimazole are pharmaceuticals that are of particular concern, increasingly being detected in surface water and soils across Europe and are toxic and mobile in the aquatic environment.

This project aims to fill a research gap in the lack of predictive understanding of pharmaceutical transformation and degradation; high resolution analytical methods have been developed for in silico identification of pharmaceuticals and their TPs but do not provide information about the mechanisms or pathways of transformation. It will determine the transformation rate of prioritised compounds (including those in the EU WFD “watch list”) and investigate their transformation pathways to help gain a better understanding of their fate in river water. The microbial and chemical data derived from these studies will be used to create a machine learning based model that can help predict the biotransformation of pharmaceuticals in river water.

Suspect screening in high resolution mass spectrometry (HRMS) has been used to detect and identify pharmaceuticals and their TPs in wastewater impacted rivers, as well as the use of online predictive transformation tools such as BioTransformer and EAWAG-Biocatalysis/Biodegradation Database/ Pathway Prediction System (EAWAG—BBD/PPS). Compounds of interest to be shortlisted were prioritised in terms of prevalence and by risk quotient which included carbamazepine and venlafaxine. Future analysis will look at targeted quantitative analysis on spiked river water and sterile artificial freshwater in aerated glass containers will be conducted using liquid chromatography tandem mass spectrometry (LC-MS/MS) to measure the amount of transformation of selected pharmaceuticals in river water over time up to 6 months (using at least two environmentally relevant concentration levels of 200 and 2000 ng/L).

### 3.12.P-Th214 The Fate of Trimethoprim, Caffeine and Sulfamethoxazole during Sub-critical Water Regeneration of Activated Carbon

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Throughout the years, wastewater treatment plants have been adding to tertiary treatments a polishing step aimed at removing/degrading micropollutants (e.g. pharmaceuticals). The most common treatments are ozonation and activated carbon. Ozonation is efficient at degrading pharmaceuticals however its byproduct could be more harmful than the initial molecules and create as well higher amount of ammonia enhancing eutrophication.

Activated carbons instead adsorb and concentrates micropollutants on its surface and can then be discarded or regenerated. Chemical and thermal regeneration techniques are often used however they require either the use of chemicals (creating more potential pollution) or are energy intensive. In our study, we instead used hydrothermal technology by degrading pharmaceuticals adsorbed on spent carbonaceous material under sub-critical water. We therefore chose to degrade three pharmaceuticals (Trimethoprim, sulfamethoxazole, caffeine) and characterize their transformation products under hydrothermal conditions between 160 to 280°C in water and then with the presence of activated carbon. The extracts were analyzed in LC-MS-QTOF on HILIC and C18 columns. For single compound degradation, trimethoprim had a high amount of transformation products (>10) compared to caffeine and sulfamethoxazole (<4). Most of these transformation products had hydroxylated substitutions or additions and at temperature higher than 200°C there has been an increase of smaller transformation products as well as an increase in partial mineralization at 240°C and above. This study also compares the transformation products observed with the ones produced in presence of activated carbons to determine if the transformation products are re-adsorbed on the surface of the char or stay in the water phase after several cycles of regeneration.

### 3.12.P-Th215 Assessment of the Potential Biotransformation of Pharmaceuticals in Zebrafish Embryos, Utilizing LC-HRMS Techniques

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Pharmaceuticals and their transformation products (TPs) are widely detected in the aquatic environment and may bioaccumulate in aquatic organisms. Zebrafish (*Danio rerio*) embryo (ZFE) has emerged as a powerful alternative model-organism in ecotoxicological studies for evaluating the effects of xenobiotics in aquatic organisms. Biotransformation constitutes an important factor in toxicokinetic studies, since sometimes, metabolites may exhibit higher toxicity than the parent compound. Identification

of biotransformation products (bio-TPs) is a challenging task, and the lack of available reference standards makes it more difficult. LC-HRMS offers a powerful approach to detect and identify these bio-TPs. Despite HRMS high applicability, separation of isomeric bio-TPs is not always possible. Hydrophilic interaction liquid chromatography (HILIC) could be used as a complementary chromatographic mode for orthogonal identification of bio-TPs. Additionally, Trapped Ion Mobility (TIMS) it is a very promising technology for the separation of isomers and could provide additional experimental evidence to enhance the identification confidence.

The aim of this study was to investigate the biotransformation capacity of ZFE exposed to different pharmaceuticals and to identify the tentative bio-TPs, utilizing LC-HRMS. Furthermore, the added value of TIMS was investigated for the identification of bio-TPs and the separation of isomers.

For the extraction of pharmaceuticals in ZFE samples, organic solvents were added, and a bead-beating homogenization process was followed. The ZFE extracts were analyzed by reversed phase liquid chromatography (RPLC) and HILIC, in both positive and negative ionization, by LC-ESI-QTOF. The extracts were also analysed with LC-TIMS-TOF-MS to introduce an additional dimension of separation, and to acquire more “clear” mass spectra. Target-screening approach was followed for the identification of the parent compounds, whereas the identification of tentative bio-TPs was performed through in-house developed suspect and non-target screening workflows. Collision cross section (CCS) values were used as additional experimental evidence for the identification.

Overall, bio-TPs from both oxidative and conjugative metabolic reactions were identified. The analytical data of this study, highlight that information in two orthogonal chromatographic techniques in combination with CCS values provided additional evidence to support the identification of bio-TPs.

### 3.12.P-Th216 Assessment of Carbamazepine Uptake and Metabolisation by *Lavandula dentata*, *Juncus Sp.* and *Salicornia europaea* Using Semi-targeted Computational Analysis Tool

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Pharmaceutical products are the emerging contaminants that probably have aroused the most interesting in the last decade because they are biological active substances, and most of their degradation and metabolic products have also biological activity. Their metabolites and degradation products can persist for a long time in the environment being even more toxic than the original compound. The scientific community is therefore concerned about their dangerousness on all kind of ecosystems and their possible harmful effects on human health. In this work, the use of a computational tool have been used for the assessment of carbamazepine metabolism pathways in plants subjected to uptake assays.

Thus, three plants with high occurrence in the natural protected area of Doñana National Park (Spain) have been irrigated with three aqueous carbamazepine solutions (10 ng·mL<sup>-1</sup>, 700 ng·mL<sup>-1</sup> and 10 µg·mL<sup>-1</sup>) and periodically, stems, leaves and roots were sampled and analysed. Total carbamazepine uptakes through the assay was evaluated and discussed showing clear dependence on plant type/part, irrigation concentration solution and assay time. In addition, a chromatographic analysis using a quadrupole- time-of-flight (Q-TOF) mass spectrometer was conducted, recording the simultaneous low- and high-fragmentation mass spectra to further computational processing using Metabolynx<sup>TM</sup> software which predicts metabolic pathways for possible ‘expected’ and ‘unexpected’ metabolites according to their *m/z* and fragmentation patterns. Several possible carbamazepine metabolites corresponding to various metabolism pathways have been identified and, where possible, their relative abundance have been discussed. Confirmation was carried out by analysing the high-energy spectra obtained at the characteristic retention time and the fragmentation patterns obtained for each compound.

The use of computational tools like Metabolynx<sup>TM</sup> have been demonstrated useful for studies that involve the search of metabolites and/or the confirmation of metabolism pathways in the studied plants.

### 3.13.A Human and Veterinary Pharmaceuticals in the Environment – Risk, Prioritisation & New Insights

#### 3.13.A.T-01 Data Gap-driven Prioritization of Active Pharmaceutical Ingredients Based on Predicted Environmental Risk

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The emission of active pharmaceutical ingredients (APIs) to the environment is an issue of increasing concern. In the European Union, an environmental risk assessment (ERA) for marketing authorization applications of medicinal products is mandatory since 2006. A comprehensive ERA is lacking for most medicines approved prior to 2006, and many pharmaceuticals have hardly any ecotoxicological data associated with them. This makes it difficult to establish their true environmental risk.

Performing ERA studies on more than 1500 APIs is unfeasible, as it would require an unacceptable number of in vivo studies and a considerable amount of time and economical resources. Considering the ethical, technical and financial limits, there is a need to identify high-priority APIs based on their predicted environmental risk and relevant data gaps.

The different approaches that can be adopted to prioritize APIs include the exposure-based approach, the hazard-based approach, and the risk-based approach. In this work, we followed a risk-based prioritization approach, combining the environmental exposure concentration (measured or predicted environmental concentration; MEC or PEC, respectively) with the Predicted No Effect Concentration (PNEC).

We collected ERA data for 1402 APIs and used different approaches to estimate exposure and effects. By combining this information, we calculated the risk quotients and developed six risk rankings including more than 1000 APIs of human medicinal



products authorized in Europe. We monitored the data availability of the top-ranking APIs from the three most relevant rankings. Finally, we identified 15 test candidates with limited ERA data that are likely to pose a potential risk to the environment, so that additional ERA data can be gathered and a more detailed ERA can be performed.

### 3.13.A.T-02 Prioritisation of Companion Animal Parasiticides in UK Rivers

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Companion animal parasiticide products are widely used to treat and prevent parasitic infestations. However, the ecotoxicological risks of these products has likely been insufficiently considered in existing regulatory risk assessments due to the assumption that exposure is negligible. With approximately 20 million cats and dogs in the UK, many being treated regularly with parasiticides, this assumption may be incorrect. Research focused on a wider range of APIs and determining an understanding of the levels of exposure and thus potential risk is necessary. Therefore, the aim of this research is to predict environmental concentrations of APIs used as companion animal parasiticides to enable the future prioritisation of these active substances based on their potential environmental risk profiles. A review of current available companion animal parasiticide products used for cats and dogs was conducted to determine the current available APIs in use within the UK. Predicted Environmental Concentrations (PECs) were then estimated for two exposure pathways: down the drain exposure and a diffuse exposure, through using simple algorithms and assumptions. A total of 124 parasiticide products were found to be in use within the UK, each containing one or more APIs. Classes of active ingredients identified within the products included; neonicotinoids, isoxazolines, macrocyclic lactones and phenylpyrazoles, with 31 APIs contained within the companion animal products. PECs were estimated for all 31 APIs for both down the drain and diffuse exposure pathways. Predicted concentrations arising from down the drain and diffuse emission pathway ranged from 0 to 42.37 ng/l with imidacloprid, praziquantel, fenbendazole and fluralaner predicted to occur at the highest concentrations. Through this work on predicting environmental concentrations of APIs used within parasiticide products for different pathways, work is ongoing to produce a relative ranking of all 31 of these active substances with the potential greatest environmental risk. Therefore, enabling further research to be conducted reducing existing data gaps through focusing on specific APIs of potential concern.

### 3.13.A.T-03 Regional Scale Modelling of Pharmaceutical Pollution in Rivers by Integrating Rural and Urban Sources

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Contaminants of emerging concern (CECs) can threaten aquatic ecosystems and human health. Both rural and urban areas are main sources of CECs to the environment. In rural areas, veterinary pharmaceuticals (VPs) are used to prevent diseases and protect the health of farm animals. The excrements of medicated animals are spread as manure to agricultural lands, where, after rainfall, VPs can be mobilized and reach surface waters through runoff. In urban areas, pharmaceuticals excreted by humans are collected in sewage systems and are only partially removed in wastewater treatment plants (WWTPs). Eventually, pharmaceuticals can reach surface waters through discharge of WWTP effluent. Currently, most of the predictive models only consider one source type, e.g. WWTPs or agricultural land. This limits their prediction performance since many CECs are being emitted by multiple source types. Therefore, this study aims to integrate urban and rural sources of CECs in one regional water quality assessment.

We predicted the concentration of CECs in the Eem river basin, the Netherlands, given land-use data combined with hydrological modeling. This allows for integrated evaluation of rural and urban emissions. These emissions were predicted with models developed within the context of the SUSPECT project (<https://cec-partnership.nl/web/index.php/projects/suspect>). CECs exposures were predicted with the Dutch National Water Quality Model where WWTPs emissions were included as point sources and rural emissions as diffuse sources. The temporal resolution of the model hydrology is seasonal. This is key to analyze the temporal variation of concentration due to manuring of agricultural lands which mainly occurs in Spring.

Predicted concentrations were successfully compared to measured concentrations taken in the SUSPECT project and from the database of the KIWK project ([www.kennisimpulswaterkwaliteit.nl](http://www.kennisimpulswaterkwaliteit.nl)) for 6 compounds: carbamazepine and fipronil (only urban sources) and trimethoprim, sulfamethoxazole, permethrin and dexamethasone (urban and rural sources).

The model train demonstrated to be a useful tool to disentangle the contribution from rural and urban sources which in different seasons may have different predominance. Our modelling approach can be generalized to land uses of the whole of the Netherlands. The land-use based predictive approach is a novel, practical tool to support decision making to reach the EU-WFD targets for the regional scales.

### 3.13.A.T-04 Modelling Multitudes of Pharmaceuticals in the Global River System at High Spatial Resolution to Prioritize Substances that Cause the Highest Risk

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As an alternative to resource-consuming extensive monitoring, contaminant fate models may be used to provide information to support the development of targeted local monitoring schemes in regions of highest exposure to pharmaceuticals in the environment as well as the prioritization of substances that require further investigation. In this work, a global contaminant fate

model (called HydroFATE) was developed with the objective of estimating the concentration of contaminants of emerging concern (including pharmaceuticals) in the global river network at a high spatial resolution (500 m). The structure of HydroFATE is based on vector routing, which besides its spatial precision being higher than in global pixel-based models, it is also faster to process. This key aspect allows for more complex analyses, including repeated execution of multiple substances and different scenarios in a short period of time, making HydroFATE a capable tool to inform on the prioritization of substances.

In the model, the contaminant emission is calculated based on consumption per capita and population density. Then, the contaminant loads of treated or untreated wastewaters are reduced either by centralized or decentralized wastewater treatment, by natural attenuation in soils and runoff, and/or by decay processes in rivers and lakes. HydroFATE was validated using measurements and applied to estimate the concentrations of the 40 most widely used antibiotics in households worldwide. These concentrations were compared to established no-effect thresholds of environmental exposure to detect potential risk. We found that the largest extent of rivers with high exposure are in Southeast Asia, the most densely populated region in the world. The main contributors of exposure were found to be amoxicillin, ceftriaxone, and cefixime.

In summary, HydroFATE combines information on river characteristics and connectivity, known characteristics of substances and their behaviour in the aquatic environment, domestic contaminant pathways to the river system, and water quality risk thresholds in a single global model, providing users with new holistic and quantitative information to support decision making and prioritization efforts at large scales.

### 3.13.A.T-05 Probabilistic Assessment and Prioritisation of Future Pharmaceutical Environmental Risks

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Understanding pharmaceuticals' contributions to future environmental risk requires consideration of how the environment, population and climate will evolve in the future. Forecasts of these factors are uncertain, and will become more so further into the future – making probabilistic environmental risk assessment, which retains uncertainty, a valuable tool for exploring future risk. We present an Object-Oriented Bayesian network for the prediction and prioritisation of environmental risk posed by six Active Pharmaceutical Ingredients (APIs) in Norway, across two time points (2020 and 2050), eleven counties, three population (low, main, high), and four wastewater treatment (WWT) scenarios (current, upgrade of all to at least secondary, upgrade to tertiary, best possible removal). Sales weights of APIs were predicted by year and population from historic sales, and used to predict environmental concentrations in WWT influent, effluent, and surface waters. Surface water Risk Quotients (RQ) per API were predicted under each scenario, and a Sum of RQ and joint probability of RQ threshold exceedance calculated across the 6 APIs. We found that in 2020, predicted SumRQ was highest in urban counties with higher population density and better wastewater treatment infrastructure, and lower in rural counties. By 2050, risk was consistent across urban and rural counties, but lower in peri-urban counties.

Comparing Sum of RQ in rural and urban counties, and Norway in 2050 (default population scenario), risk is expected to increase if WWT is upgraded to secondary or tertiary due to lower removal of risk-driving APIs under these treatments. Risk in urban counties is mostly unaffected due to the efficient technologies already used in WWT. However, both in rural counties and across the whole country, switching to the best available treatment technology is expected to lower overall risk.

Under low, main and high population scenarios Norway's population could range from 4.5 to 7.5 million, but little effect was seen on risk. Perhaps as population growth drives both modelled sales and dilution, Sum RQ across the whole country was predicted to drop in the low growth scenario and be unaffected by high growth.

This work represents a useful case study for the use of a spatial and temporally discretised Bayesian network for prediction of environmental risk and identification of APIs driving risk distributions with the full consideration and transparent presentation of uncertainty.

### 3.13.B Human and Veterinary Pharmaceuticals in the Environment – Risk, Prioritisation & New Insights

#### 3.13.B.T-01 Effect of Lowering pH and Increasing Temperature on Toxicity of Sulfamethoxazole to *Daphnia magna*

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The problem of global warming is intensively studied. However, little is said about its impact on the toxicity of pharmaceuticals, new emerging pollutants with not fully understood environmental impact. To date, there is little knowledge of the problem and continued experimental research is required. Scientists stress that the problem of global warming can result in changes in the population of organisms and even lead to their extinction. In addition, the presence of pharmaceuticals, may intensify the negative effects of global warming, hence in-depth studies should be conducted. Therefore, it was decided to study the toxicity of sulfamethoxazole (antibiotic widely used in veterinary medicine) under the influence of predicted climatic changes: firstly, under the increase in temperature, secondly under the decrease in pH, finally under the influence of a combination of both stressors. A crustacean *Daphnia magna* was taken as target water organism. Acute and chronic toxicity tests were carried out in accordance with OECD guidelines 202 and 211. These experiments allowed the determination of EC<sub>50</sub> and NOEC and LOEC parameters for sulfamethoxazole. High-performance liquid chromatography with a DAD detector was used as the quantification technique during the experiments to check the actual sulfamethoxazole concentration. These studies, combined with available literature data, concluded that the toxicity of sulfonamides increases with increasing temperature and decreasing pH. In addition, it was shown that exposure of *D. magna* to synergistic effects of both stressors causes the greatest toxic effect. Chronic toxicological tests showed a negative effect of low concentrations of sulfamethoxazole on *D. magna* reproduction, which is important due to the

presence of sulfamethoxazole in the environment. Thus, this research contributes to the understanding of the broad problem of the effect of pharmaceutical toxicity on aquatic organisms.

### **3.13.B.T-02 Molecular, Biochemical and Cellular Effects of Single and Combined Pharmaceuticals in *Mytilus galloprovincialis***

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The environmental consequences of pharmaceuticals in aquatic environments are now recognized as a real problem due to a global-scale distribution and detrimental outcomes for non-target organisms. The capability of marine species to simultaneously accumulate various typologies of pharmaceuticals has recently been shown, along with the onset of deleterious effects caused by single active ingredients and affecting the organismal health status. Despite this evidence, mechanisms of interactions and adverse outcomes of low levels of pharmaceuticals mixtures have received so far limited attention. In this respect, the present study was aimed to investigate interactive effects of antidepressants, lipid lowering agents, antiepileptics, cardiovascular and non-steroidal anti-inflammatory drugs in the Mediterranean mussels *Mytilus galloprovincialis*. A series of *in-vivo* experimental plans was set up exposing mussels for up to 30 days to various environmentally realistic concentrations (0.5-1 µg/L) of selected pharmaceuticals both alone and in mixtures. Analyses of drugs bioaccumulation were integrated with early molecular responses measured as changes in transcriptomic profile, functional analyses at cellular level through a large panel of ecotoxicological biomarkers including alterations of the immune system, variations of oxidative stress biomarkers, onset of genotoxic damage and changes in distribution of lipids, proteins and carbohydrates. The overall results were finally integrated through a quantitative Weight Of Evidence (WOE) model that elaborates specific hazard indices based on the number, magnitude and toxicological relevance of observed responses. Interactive and competing mechanisms among tested drugs modulated bioaccumulation and biological responses, allowing to further measure changes in transcriptomic profile and the onset of detrimental effects, with both synergistic and antagonistic interactions depending on investigated mixture and endpoint. The elaboration of overall results through the quantitative WOE model provided new perspectives for a more comprehensive risk assessment of environmental mixtures of pharmaceuticals.

### **3.13.B.T-03 A Year-long Study of the Occurrence and Risk Assessment of Over 140 Contaminants of Emerging Concern in a Range of Aquatic Matrices in the Republic of Ireland**

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Contaminants of emerging concern (CECs) are increasingly being shown to occur in water samples across the world, and their risks require further investigation. The breadth of such compounds is increasing and there is a constant need for reliable analytical methods for identification and their determination. Despite being a developed country in the European Union (EU), knowledge of the nature and extent of contamination of water bodies with CECs in Ireland is limited.

In this study a broad range of >140 CECs including pharmaceuticals, pesticides, and personal care products were monitored monthly in influent and effluent wastewater and receiving surface waters at a rural and urbanised area over a year period. Across all samples, 58 CECs were detected, where venlafaxine, currently under the “EU-Water Framework Directive Watch List”, obtained the highest concentration in influent overall (8,273 ng·L<sup>-1</sup>). Wastewater treatment efficiency was assessed, and high removals, >98%, in both areas were observed for compounds such as azithromycin and EE2. A further reduction from the CEC effluent concentration compared to surface waters was observed (≥~93%). Correlation between surface air temperature and CEC wastewater concentrations were also investigated. Lastly, an environmental risk assessment was performed using risk quotients (RQs), showing an ERQ magnitude lower in surface waters when compared to effluent.

For the first time, the temporal and spatial occurrence of >140 CECs were monitored in the aquatic environment and WWTPs over a period of a year at two sites in Ireland. Several quantifiable and unmonitored chemical residues were present in these locations, but their risk to aquatic wildlife was lower in comparison to other developed states. The monitoring data will enable risk-based prioritisation for monitoring these pollutants for Irish water catchments, providing a comprehensive insight into the occurrence, fate and impact of CECs in Irish waters.

### **3.13.B.T-04 Occurrence of Emerging Contaminants in Wastewater and Groundwater in Botswana**

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Emerging contaminants (ECs) represent a wide range of chemical compounds and their occurrence in the environment is a global concern; however, little is known about their occurrence and exposure in many parts of the world including southern Africa. It is essential that the levels of ECs are monitored closely as many of these compounds, e.g. pharmaceuticals, are designed to produce effects at very low concentrations and exposure should be limited in order to mitigate potentially harmful ecotoxicological impacts.

Monitoring of ECs has been possible due to increased capability of analytical instrumentation and methodology such as non-targeted and suspect screening through the use of chromatographic separation techniques coupled with High Resolution Mass Spectrometry (HRMS). However, One significant challenge in environmental monitoring of ECs is that investigations are limited to developed countries due to the accessibility to analytical instrumentation. Thus, we evaluated wastewater treatment plants as a source of ECs in Gaborone, Botswana as well as ECs in groundwater using HRMS with non-targeted and suspect screening methods.

Screening for 5942 biologically active compounds, a total of 28 compounds were detected at least once, including 26 pharmaceuticals and two illicit drugs (2-Ethylmethcathinone and 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol) that have been a source of concern for authorities in Botswana recently. This represents the first detection of illicit drugs in wastewater in Botswana. The highest number of detections were in influent wastewater (26 ECs) followed by effluent wastewater (10 ECs) and lastly, groundwater (4 ECs).

17 emerging contaminants detected in the influent water were not detected in the effluent waters, suggesting some level of reduced emissions from wastewater treatment. The remaining nine chemicals were detected at lower intensities, a qualitative proxy for incomplete removal, in the effluent samples. Sulfamethoxazole was detected in wastewater effluent and non-target analysis revealed its human metabolite, N4-acetylsulfamethoxazole, was present in influent wastewater samples. Two anti-retroviral compounds (abacavir and tenofovir) were detected in influent and effluent sources. This confirms that wastewater treatment plants are a source of biologically active chemical pollution to the environment in Botswana and will help inform prioritization efforts for monitoring and remediation that is protective of these ecosystems.

### **3.13.B.T-05 Presence & Removal of Psychopharmaceuticals & Transformation Products from Dutch Wastewater Streams**

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Psychopharmaceuticals are primarily used to treat psychological disorders, as well as other conditions relating to the nervous system and are known to affect non-target organisms in the environment at very low concentrations. The occurrence of many psychopharmaceuticals in the water cycle remains an understudied topic, with data lacking for many compounds. Therefore, the current study aimed to provide insights into the presence and removal of psychopharmaceuticals and their transformation products in Dutch wastewater streams employing target and suspect analysis. 51 target compounds were selected based on high (Dutch) prescription or high environmental risk indicated in literature. A suspect list was also generated based on the 200 psychopharmaceuticals prescribed in the Netherlands, plus 6000 predicted transformation products of these compounds. Solid Phase Extraction (SPE) was carried out on 24h composite samples from the Wastewater Treatment Plants (WWTPs) of 6 Dutch cities. From each location both effluent and influent samples were collected from at least 7 days. Separation and detection were performed with UHPLC-qTOF-HRMS. Suspect screening was performed using the open-source platform PatRoon 2.0, and select suspects with high structural similarity to targets underwent semi-quantification.

Based on initial results, 34 target compounds were detected in the effluents ranging from 1 – 2,500 ng/L, while 36 were detected in the influents, ranging from 1 – 250,000 ng/L. Ongoing analysis shows that suspect compounds, including both parent compounds and transformation products, are detected in both types of water samples. Since many suspect compounds have target analogues, these could therefore be semi-quantified, indicating that transformation products are often found in higher concentrations than parent compounds.

Utilising PatRoon 2.0 and semi-quantification in tandem allowed to quantify more compounds than previous studies. Moreover, we revealed the presence of several transformation products, which are often overlooked. The results of the present study highlight that wastewater matrices contain abundant numbers of psychopharmaceutical transformation products and, like some of the parent compounds, these are not removed sufficiently even from modern Dutch WWTPs. Analysis is still ongoing, but it is likely to be concluded that modern WWTPs require upgrades to lower the quantities of these compounds released into the aquatic environment.

### **3.13.C Human and Veterinary Pharmaceuticals in the Environment – Risk, Prioritisation & New Insights**

#### **3.13.C.T-01 Contribution of Septic Tanks to Pharmaceutical Concentrations in Rivers**

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Pharmaceuticals are emerging contaminants in the environment. Due to their incomplete removal in conventional wastewater treatment works (WWTW) and their ubiquitous presence in influent, treated wastewater discharges are considered the main entry source of pharmaceuticals into the environment. So far, research has focused on centralised WWTW and the receiving surface waters. However, it is conservatively estimated that 7% of the Scottish population, are served by a septic tank. Septic tanks are typically located in rural and semi-urban areas and treat wastewater of individual or small groups of houses.

Five septic tanks (with 217 – 475 PE) discharging to four different rivers in Scotland were monitored for 69 pharmaceuticals between October 2021 and September 2022. A seasonal effect on the sum of concentrations of pharmaceuticals in influent and effluent can be observed. In all septic tanks, the lowest concentrations were reported in February during heavy rainfall. Findings indicate that pharmaceutical concentrations in septic tank effluents can exceed concentrations typically reported in centralised WWTW effluents. For instance, venlafaxine was determined at concentrations up to 1.2  $\mu\text{g L}^{-1}$  in septic tank effluent, approximately two-times higher than previously reported maximum concentrations in the UK. Initial data has shown correlations between the concentrations of some pharmaceuticals and sanitary determinands. For instance, metformin and the biological oxygen demand have a correlation coefficient of 0.9 in septic tank effluent ( $n = 30$ ).

River water samples were collected quarterly upstream ( $n = 20$ ) and downstream ( $n = 20$ ) of the effluent discharge point. The contribution of the septic tanks to the sum of pharmaceuticals detected in the river varies from no difference between upstream and downstream concentrations to an increase of 95%. Detection frequencies vary between the different analytes. The frequently consumed drug paracetamol was detected in 75% of the upstream samples at concentrations from 0.3 to 404  $\text{ng L}^{-1}$ , and in 95% of the downstream samples at concentrations from 0.3 to 1548  $\text{ng L}^{-1}$ . In contrast, the beta-blocker atenolol was not detected

downstream, and in 25% of the upstream samples at concentrations from 0.1 to 3.4 ng L<sup>-1</sup>. Risk quotients (RQ) were calculated from the environmental concentration and the predicted no effect concentration. The highest RQ was found for ibuprofen (RQ = 13) in a downstream sample in August.

### **3.13.C.T-02 Sewage Overflows and Historical Landfills Affecting Pharmaceutical Pollution in Scottish Rivers: Spatiotemporal Distribution and Hazards**

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Active pharmaceutical ingredients (APIs) are known contaminants in the aquatic environment linked to sources including sewage treatment effluent, combined sewage overflows (CSO), among others. For example, recent evidence suggests that landfills, particularly those built prior to regulatory requirements for base lining systems, may also be a source. As APIs are designed to interact with highly conserved molecular receptors, their occurrence is of ecological concern.

Despite decades of research, few studies monitor APIs across large geographical catchments and/or collect samples over long time periods (e.g., >6 months). Here we present a year-long study of APIs in 11 rivers of the Firth of Forth catchment (Scotland, UK) representing the chemical fingerprint of approximately 1.5 million people (27% of the Scottish population). Duplicate river water grab samples (n=26 sites and 624 total samples) were obtained monthly for 1 year up-/downstream of potential sources of organic contaminants (e.g., sewage treatment plants, agricultural, poultry rearing, landfill and industrial areas). Targeted analysis occurred for 70 analytes using high performance liquid chromatography tandem mass spectrometry generating 43,680 chemical determinations.

APIs were quantified in all but 1 sampling site with the most contaminated rivers being those in the most highly populated regions of the catchment surrounding the city of Edinburgh. In total, 33 analytes were detected. The most contaminated site was consistently a location on the river Carron (Grangemouth) 7 km downstream from a historic landfill which erodes into the river during high-flow conditions. Here, cumulative concentrations of the studied contaminants (mean=9097ng/L) were typically 2-3-fold higher than the next most contaminated location. Highest concentrations were observed in July and August which coincided with low-flow conditions or during periods of suspected CSO events. Contaminants with >75% detection frequency included caffeine, carbamazepine, codeine and gabapentin. Comparison of the measured concentrations to apical and non-apical predicted no effect concentrations (PNECs) in the literature showed that concentrations of amitriptyline, diltiazem, paracetamol and metformin exceeded non-apical PNECs in 25%, 14%, 11% and 3% of the collected samples respectively. This work highlights the effects of different API sources on environmental concentrations and ecological risk across large spatial and temporal scales.

### **3.13.C.T-03 Seasonal Speciation of Pharmaceuticals Within a Full-Scale Free Water Surface Constructed Wetland in England**

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Treated wastewater is a major source of pharmaceuticals and their metabolites in the aquatic environment in the UK due to their widespread use. Conventional wastewater treatment systems are not designed to effectively remove these organic micropollutants. Consequently, complex mixtures are consistently released into the environment at low concentrations posing potential risks to aquatic ecosystems and human health. As a tertiary step in wastewater treatment, constructed wetlands offer a low-carbon treatment solution for removal of pharmaceuticals and other chemicals of emerging concern. Adoption of this nature-based technology in the UK is limited, and most of the current literature refers to pilot scale and mesocosm systems. This research assesses seasonal speciation of pharmaceuticals within a full-scale free water surface constructed wetland in South-West England which receives effluent from a rural wastewater treatment plant (2,000 population equivalent). The wetland was installed by Wessex Water in 2020 to comply with obligations under the UK Water Industry National Environment Programme.

Three sampling campaigns were carried out during the first year of operation. Water and sediment samples were prepared by solid phase extraction and the QuEChERS method, prior to analysis by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Of 139 chemical targets, > 80 were identified in wetland water samples including pharmaceuticals and their metabolites, illicit drugs, and lifestyle chemicals. Efficiency of removal from the aqueous phase is compound dependant, with ≥ 90% removal of some compounds (e.g., quinolone antibiotics and statins) and poor removal or increase of others (e.g., macrolide antibiotics and nicotine). As expected, the wetland appears most efficient in the summer months. Biodegradation and photodegradation are considered dominant removal mechanisms based on seasonal variability in removal efficiencies and metabolites. Partitioning to sediments appears to have a minor role, with fewer compounds quantifiable in sediments. Some concentrations exceeded literature predicted no effect concentrations in wetland effluent, but nevertheless the wetland acts to significantly reduce dissemination of pharmaceuticals from treated wastewater into the wider environment. Detailed assessment of removal mechanisms may be used to determine optimal operational parameters for similar systems installed in the UK in future.

### **3.13.C.T-04 Environmental Safety of Parasitocidal Veterinary Medicinal Products for Companion Animals from a European Regulatory Perspective: Challenges and Knowledge Gaps**

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In the last decades, much effort has been invested in the development of suitable active substances and well-adapted medicinal products to improve user and animal safety, as well as the efficacy of parasitocidal medicinal products for cats and dogs. At the same time, research into the environmental safety of such products has been neglected, in spite of their evident insecticidal and acaricidal effects. The assumption of the guideline on environmental risk assessment (VICH GL 6) is that the treatment of companion animals only leads to negligible environmental exposure and thus no experimental Phase II risk assessment is required. This presentation will give background information on regulatory aspects associated with the topic of the environmental safety of (ecto-) parasitocidal veterinary medicines for companion animals and spotlight the major scientific knowledge gaps that lead to the most significant regulatory challenges in the EU.

Major knowledge gaps identified that hamper regulatory decision making and that prevent from the performance of a quantitative and product based risk assessment are related to: (i) the lack of robust environmental exposure models (PEC derivation), (ii) real world evidence from aquatic hot-spots (e.g. dog swimming areas or in the vicinity of WWTP influents and effluents), (iii) real world evidence from urban and peri-urban terrestrial ecosystems exposed to excreta from treated animals, and (iv) data on the environmental fate and behaviour (including sewage sludge and sediment) of active substances used. Such knowledge gaps exist for most active substances used in ectoparasitocidal veterinary medicines for companion animals, however, are most pronounced for newly developed substances, many of which are persistent and can be classified as PFAS.

This regulatory perspective may provide the scientific community with important considerations for developing targeted research strategies to investigate the environmental concentrations, behaviour and safety of parasitocidal veterinary substances for companion animals, as well as suggestions for environmental modelling parameters.

### **3.13.C.T-05 Proposal for Strengthening the ERA Whilst Maintaining Access to Human Medicinal Products – The Extended ERA Approach**

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One of the unintended results of delivering medicines to patients is that the Active Pharmaceutical Ingredients (APIs) contained in medicines can enter the environment through excretion after patient use. It is therefore essential to assess the potential impact APIs can have on the environment. To that end, since 2006, it is mandatory to include an **environmental risk assessment (ERA)** as part of regulatory marketing authorisation application (MAA).

The current ERA has been invaluable to assessing the environmental risk of APIs since 2006. Learnings from the assessment of potential environmental risks (both by industry and by regulators) over the years show that further improvements could be made. With the ongoing revision of the EU-Pharmaceutical legislation, (Directive 2001/83 EC), there is an opportunity to address and implement such improvements. This presentation outlines the extended ERA (eERA) approach to addressing the concerns with the current process. Critically, this approach would extend and strengthen the existing ERA framework beyond the initial, single medicinal product-based assessment in the EU, without compromising patient access to medicinal products.

The eERA approach proposed here by the three pharmaceutical industry trade associations (EFPIA, AESGP and Medicines for Europe) is designed to address the current challenges with ERA and strengthen the ERA process in the EU. Some of these challenges with the current ERA approach identified and proposed to be addressed by the eERA are summarised below: 1) Insufficient transparency and accessibility to environmental data and risk assessments of APIs 2) Potential for duplication of testing 3) Potential for conflicting risk conclusions 4) Calls to include ERA conclusions in the MAA evaluation process 5) Environmental risk resolution burden on the 'last to market'

The eERA addresses these challenges and the risk of APIs from three different perspectives: Situation 1 – new innovative APIs fulfilling an unmet need in patients; Situation 2 – off-patent APIs or APIs post data exclusivity; and Situation 3 – so-called legacy APIs on the market (marketed before 2006) without sufficient environmental data to conclude on risk. The eERA approach presented demonstrates numerous benefits in overcoming these limitations, while not compromising patient access to medicinal products.

### **3.13.P Human and Veterinary Pharmaceuticals in the Environment – Risk, Prioritisation & New Insights**

#### **3.13.P-We119 Prioritisation of Pharmaceuticals for Assessment of Exposure and Risks to Terrestrial Ecosystems**

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Concerns over environmental exposure to active pharmaceutical ingredients (APIs) has been increasingly reported in the literature, with the aquatic compartment being the predominant focus. Many attempts have been made to prioritise APIs based on their potential to impact the aquatic environment. Less attention has, however, been given to the prioritisation of APIs in the terrestrial environment, despite being released to terrestrial systems during sewage sludge amendment or wastewater irrigation. Here, APIs were prioritised based on exposure and risk to terrestrial ecosystems using data from the NHS England 2018

Prescription Cost Analysis. Compounds assumed to be in use by approximately 1% of the population were considered within the prioritisation, with predicted environmental concentrations (PECs) in wastewater being generated and refined using API-specific excretion rates (where available). The European Medicine Agency (EMA) threshold of 0.1 µg/L<sup>-1</sup> in wastewater effluent was used

to further focus the list of study APIs, with an additional safety factor applied to hormones and anti-cancer drugs. PECs for sludge and wastewater effluent were then obtained which were subsequently used to derive soil and porewater PECs. Exposure predictions were then combined with predictions of ecotoxicity and bioaccumulation to derive risk quotients for soil invertebrates and birds and mammals. APIs were then ranked by exposure and risk to terrestrial ecosystems. From the initial screening of the top 217 compounds, Lactulose, Paracetamol and Metformin were the most prescribed in terms of mass, with antibacterial and antidiabetic drug classes appearing most frequently within the prioritisation list. Considering parent compound excretion, Lactulose, Metformin and Gabapentin had the highest PECs in influent, at 1.42, 0.21 and 0.05 mgL<sup>-1</sup> respectively. Montelukast and Atorvastatin however, showed the greatest risk of bioaccumulation in earthworms, with predicted Log bioconcentration factors of 7.42 and 4.82. The toxicity ranking is ongoing. Refining terrestrial risk assessment based on exposure, through consumption and resulting PECs, provides a useful method in which to prioritise pharmaceutical compounds for experimental study and model validation. The adaptability of this method to consider different consumption, wastewater treatment and both sludge and reclaimed water application scenarios, presents the means to contribute to future prioritisations in terrestrial systems.

### 3.13.P-We120 An Optimized Risk Assessment for Prioritization of Pharmaceuticals and Personal Care Products in Four Major Rivers in South Korea

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Pharmaceuticals and Personal Care Products (PPCPs) may exist in low concentrations in environmental matrixes, but pseudo-persistent and toxicity at low concentrations can lead to detrimental effects on organisms, such as endocrine disruption. In this study, an optimized aquatic ecological risk assessment of 137 PPCPs was conducted with the application of methodology developed by the NORMAN network. Prior to risk assessment, the Measured Environmental Concentration (MEC) of PPCPs was investigated via an application of multiresidue screening method using LC-HRMS in a spatiotemporal manner. Predicted No Effect Concentration (PNEC) was derived from different types of PNEC based on data availability, Environmental Quality Standards (EQS), PNEC derived from available toxicity data for aquatic species with an application of safety factor, and predicted PNEC derived from the predictive model. Among 137 PPCPs and 84 PPCPs were quantified above the LOQ with concentrations ranging from 1 ng L<sup>-1</sup> to 51 µg L<sup>-1</sup> (e.g., metformin). An optimized risk assessment was evaluated based on two indicators, the *Frequency of Exceedance* and the *Extent of Exceedance*. *Spatial Frequency of Exceedance* of the PNEC was calculated by the proportion of the number of sites with concentration exceeding PNEC and a total number of sampling sites, and 13 PPCPs posed a potential risk to aquatic organisms. Among 13 PPCPs, the proportion of clotrimazole and carbamazepine that exceeded their respective PNECs were 1 and 0.97, respectively. *The Extent of Exceedance* of the PNEC, identical to the Risk Quotient (RQ) approach, was calculated as 95th percentile upper confidence limit of all MEC values divided by the PNEC and classified them as low risk if exceedance is greater than or equal to 0.1, moderate risk for ranging from 0.1 to 1 and high risk for above 10. 18 PPCPs were identified as posing a risk, and similar to results obtained from the frequency of exceedance, carbamazepine (RQ=37.9) and clotrimazole (RQ=17.4) were classified as high risk, and 5 PPCPs were classified as moderate risk. Unlike the sub-score methods developed by NORMAN Network, values obtained from two indicators were multiplied for further prioritization. As a result, carbamazepine, clotrimazole, azithromycin, dichlorvos, flubendazole, imidacloprid, clarithromycin, galaxolide, valsartan acid, diclofenac acid, ranitidine, venlafaxine, climbazole were ranked respectively as priority compounds that posed risk to aquatic ecosystem.

### 3.13.P-We121 Prioritization, Learnings, and Strategies to Close Data Gaps of the Active Pharmaceutical Ingredient Disulfiram

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Today there are many active pharmaceutical ingredients (APIs) on the market which were registered as human medicinal products before 2006. Environmental risk assessments (ERA) were only requested by EU authorities thereafter. Hence, many APIs lack environmental data and full environmental risk assessments may be highly uncertain.

One of the aims of the IMI PREMIER project ([www.imi-premier.eu](http://www.imi-premier.eu)) is highlighting methodologies to prioritise the closing of existing data gaps for APIs without an ERA. Within the project, at least 25 APIs shall be prioritized either due to their mode of action (MoA) or risk related criteria. Ecotoxicological tests will subsequently be performed for these APIs.

Disulfiram was among the APIs which were prioritized and ranked high for risk related criteria. Some relevant and reliable data was identified in the ECHA database during a systematic literature search to identify data relevant for ERA. Disulfiram is also used in, e.g., adhesives and sealants, which explains the assessment under REACH. Only a limited number of peer-reviewed studies were found to be relevant and these were then CRED assessed for reliability. In the end, appropriate chronic aquatic toxicity data on fish was missing, but experimental data would potentially not be required when applying a decision tree developed for legacy pharmaceuticals that aims to reduce *in vivo* fish testing.

We will present the API prioritization process of disulfiram within PREMIER, learnings, and strategies to identify the benefits of closing the data gap on fish with experimental studies or substituting data.

### 3.13.P-We122 Monitoring Pharmaceuticals Consumption using PERK: A Study Utilizing Prescription Data Collected at the River Catchment Level with High Spatial Resolution

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The prescription data of selected pharmaceuticals were utilized to predict their concentrations in the wastewater influent. A combined wastewater-based epidemiology and environmental risk analysis protocol was developed. Prescription data for the catchment area is derived from England's National Health Services monthly prescription database using PrAna. Using PERK, predicted environmental concentration (PEC) of pharmaceuticals were estimated and validated against measured environmental concentration (MEC). The wastewater monitoring data obtained from five different wastewater treatment plants in the Southwest of England (WWTP A to E) were used.

The model can predict accurate results for 16 pharmaceuticals ( $0.5 < \text{PEC}/\text{MEC} < 2$ ) and acceptable results for 31 out of 36 pharmaceuticals ( $0.1 < \text{PEC}/\text{MEC} < 10$ ), according to comparisons between predicted environmental concentrations (PEC) of pharmaceuticals in wastewater influent ( $\text{PEC}_{\text{influent}}$ ) and measured environmental concentrations ( $\text{MEC}_{\text{influent}}$ ) reported in prior study. 28 of the pharmaceuticals that were studied were prescription-only medicines (POM). The model achieved accurate results for 12 pharmaceuticals and acceptably accurate results for 24 out of 28 pharmaceuticals in POM, where prescription counts are presumed to be a good indicator of use. For most of the pharmaceuticals under study at the five WWTP locations, there were similar variation between measurements and prescriptions.

Pharmaceutical removal percentage (Removal%) was calculated using measured effluent and influent concentrations. The Removal% and  $\text{PEC}_{\text{influent}}$  were used to predict the PEC values in effluent ( $\text{PEC}_{\text{effluent}}$ ). Metformin, tramadol, naproxen, and carbamazepine were the top five medicines found in wastewater at all five study sites, with values from 0.3 to >25 g/L. Due to both their high consumption and low removal rates in WWTP, the top five pharmaceuticals had  $\text{PEC}_{\text{effluent}}$  levels that were high (>4 g/L) in the wastewater effluent at WWTP D. As part of the environmental risk assessment (ERA), the PEC to predicted no effect concentrations (PNEC) ratio for several pharmaceuticals was also computed.

### 3.13.P-We123 Innovative Data Visualisation Tool to Explore Relationships Between Pharmaceutical Prescribing and Environmental Occurrence

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The presence of pharmaceutical residues in the aquatic environment is recognised internationally as an important public health and environmental issue. Pharmaceuticals are continuously introduced into effluent-receiving surface waters due to their incomplete removal within wastewater treatment plants (WWTPs). The environmental fate and risks of these residues are not fully characterised, but many compounds are of ecotoxicological and regulatory concern due to adverse effects in non-target organisms at environmentally relevant concentrations.

To develop a deeper understanding of this issue in Scotland, representatives from the environment, water and healthcare sectors formed the One Health Breakthrough Partnership. With researchers, this partnership performed a baseline assessment of pharmaceuticals in Scotland's water environment and developed the first national database combining data from regulatory monitoring, published and grey literature. The database was collated into an open-access, interactive data visualisation tool to combine national environmental data and community prescribing data on pharmaceuticals.

The environmental dataset, containing >3000 datapoints, representing 60 pharmaceuticals in 11 distinct environmental matrices, was first interrogated to explore spatial trends and environmental risk. Spatial analysis revealed relationships with population demographics, with significant monitoring gaps in rural areas of Scotland, and most monitoring targeting "high risk" locations e.g., directly downstream of WWTPs. Preliminary risk assessment, based on a holistic consideration of risk quotients, detection frequencies and prescription volumes, identified several substances with higher ecotoxicological risk in inland surface waters, and risk of driving antimicrobial resistance (based on minimum inhibitory concentrations). Environmental data has been matched to standardised quantities of drugs prescribed, by health board, GP practice and drainage operational area, enabling users to view both datasets over a range of spatial scales. Future analysis will interrogate prescription trends to explore possible relationships between prescribing and environmental occurrence of pharmaceuticals. The tool will continue to be developed and updated as data becomes available. It is envisioned to be used by stakeholders to inform the development of substance specific and location specific interventions to reduce potential risks to environmental and human health.

### 3.13.P-We124 Modeling the Sustainable Re-Use of Treated Water Containing Active Pharmaceutical Ingredients for Land Application

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Treated water is increasingly being re-used as irrigation to help achieve sustainability objectives in pharmaceutical manufacturing processes. Potential re-use of treated water can reduce discharge directly to waterbodies or sewage treatment plants and is important for water conservation. Treated water from pharmaceutical manufacturing processes may contain low levels of active pharmaceutical ingredients (APIs) and any sustainable re-use requires an understanding of the fate and transport of these compounds in the environment and their associated environmental risk.

Using a conservative screening level modeling approach, we customized USEPA's Pesticide Root Zone Model (PRZM) to simulate spray irrigation applied to established turf. Model inputs include soil, climate, and API properties, as well as the API production schedule and proposed irrigation practices at the facility. Modeled estimates of predicted environmental concentrations (PECs) of APIs in soil, surface water runoff, and leachate to groundwater were calculated by the model on a daily time step over a period of ten years and compared with environmental threshold limits. An automated optimization routine was developed to



determine PECs of an API in treated water (or the irrigation rate) appropriate for remaining below the environmental thresholds for the API. This screening level modeling approach for re-use of treated water enables a facility to potentially maximize treated water reuse, minimize treated water discharge, identify appropriate treatment technologies, and minimize potential to exceed relevant threshold levels in soil, surface water, and groundwater as a component of a sustainability objective.

### **3.13.P-We125 Human Plasma Protein Binding is a Good Predictor of Sludge Sorption For Cationic Pharmaceuticals**

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The sludge:water partitioning coefficient ( $K_{d\text{sludge}}$ ) is a key predictor of the fate of pharmaceuticals in sewage treatment plants. As such,  $K_{d\text{sludge}}$  is of great importance for the prediction of surface water concentrations and so is accurately evaluated by means of the OECD TG 106 assay for the regulatory environment risk assessment (ERA) of human pharmaceuticals. For many legacy pharmaceuticals currently on the market, measured  $K_{d\text{sludge}}$  data is not available, thus modeling their fate in sewage is often based on the octanol:water partition coefficient ( $K_{ow}$ ) even though  $K_{ow}$  is not a reliable predictor of sorption for ionizable compounds such as pharmaceuticals. While complex polyparameter models may be able to better predict sorption, a simple linear regression model would be preferable in terms of transparency and ease of implementation for regulatory purposes.

Extensive datasets gathered during drug development are worth exploring for estimating environmental fate and effects. Human plasma protein binding (HPPB) is assessed to facilitate prediction of pharmacokinetic parameters and efficacy. This assay shares conceptual similarities with sludge adsorption study, as sorption is evaluated towards an organic substrate with a net negative surface charge. Therefore, this study examined the possibility to predict  $K_{d\text{sludge}}$  from HPPB data.

HPPB data, log Dow (octanol:water ~pH 7), and  $K_{d\text{sludge}}$  data was collected for 75 pharmaceuticals, classified according to the predominant ionic species ~pH 7. Linear regression results indicate that log Dow is a reasonably good predictor of  $K_{d\text{sludge}}$  for neutral pharmaceuticals, but a rather poor predictor for cationic pharmaceuticals. However, HPPB is a better predictor of the  $K_{d\text{sludge}}$  for cationic pharmaceuticals, with an even stronger correlation than the one between log Dow and  $K_{d\text{sludge}}$  for neutral pharmaceuticals. The results of this study suggest that for cationic legacy pharmaceuticals, easily accessible HPPB data may be used to predict partitioning in sewage with reasonable accuracy. HPPB data could also be used to estimate the likelihood that a terrestrial assessment will be triggered in the ERA.

### **3.13.P-We126 Water Pollution from Pharmaceutical Use in Livestock Farming: Assessing Differences Between Livestock Types and Production Systems**

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Livestock production is considered as major source of pharmaceutical emissions to the environment. The current scientific discourse focusses on measuring and modelling emissions as well as assessing their risks. While several studies evidence the severity of pharmaceutical pollution resulting from livestock farming, differences in pollution between livestock types and production systems are largely unknown. In fact, there is no comprehensive analysis of factors influencing pharmaceutical use – the emission's source – in the diverse production systems. To address these knowledge gaps, the present research develops a framework to investigate pharmaceutical pollution from different livestock production systems. Using the framework, a first pilot assessment is conducted comparing pollution from organic and conventional cattle, pig and chicken production systems for selected indicator substances, covering antibiotics, antiparasitics, hormones and NSAIDs. Hereto novel qualitative information about influential factors for pharmaceutical use and pollution (which is used where public databases lack statistics) is combined with quantitative data (e.g. about the environmental behavior of specific substances). Both, framework development and pilot assessment base on interviews and literature. The analysis shows that factors across a pharmaceutical's entire lifecycle influence pollution. However, not all of them are livestock type or production system dependent. The pilot assessment reveals that differences in pollution potential between conventional and organic production exist, but for antibiotics, NSAIDs (and partially antiparasitics) some factors lead to higher pollution potential in conventional, others in organic systems. For hormones we identified a comparatively higher pollution potential from conventional systems. Among the indicator substance, the assessment over the entire pharmaceutical lifecycle illustrated that flubendazole in broiler production has the highest per unit impact. The framework (and insights from the pilot assessment) are useful to identify which substances, livestock types, production systems, or the combination thereof has high or low pollution potential. This knowledge can serve more sustainable agricultural management and ultimately less pharmaceutical pollution.

### **3.13.P-We127 Occurrence of Veterinary Antibiotics and Effect on the Change of Microbial Community in the Watershed**

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Recently, concern has been increased on the released veterinary antibiotics (VAs) in the environment because of adverse effect on ecosystem and production of antibiotic resistance genes (ARGs). The main purpose of this research was i) to monitor the occurrence of VAs in the surface water, soil, and sediment, and ii) to identify the change of microbial community in the sediment due to released VAs. Total of 12 VAs including four different classes (penicillins, tetracyclines, macrolides, and sulfonamides) was measured using liquid chromatography-tandem mass spectrometry (LC/MS/MS) in the surface water, soil, and sediment. Metagenomic analysis was performed using the Illumina Miseq platform to understand the change of bacterial community in sediment. The result showed that seven VAs were found at five locations, and the detected mean concentration of VAs was ranged 0.02–0.31 µg/L, 1.37–142.78 µg/kg and 3.73–9.04 µg/kg in surface water, soil and sediment samples, respectively. Change of microbial community was also observed that the most dominant species was *firmicutes* and main source of released VAs can

be effluent from the livestock manure treatment plant and the runoff from the livestock farms. In addition, the relative abundance of bacteria such as *Clostridium butyricum*, *Clostridium saudiense* and *Clostridium gasigenes* were higher in confined and dense livestock farm than in other places. Released VAs from the animal farm can change the microbial community in the watershed and further study should be necessary to verify the production of ARGs due to residual of VAs in the environment.

### 3.13.P-We128 Analysis of Pesticides, Pharmaceuticals and Personal Care Products in Drinking and Environmental Water by Direct Injection Using UPLC-MS/MS

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Monitoring for harmful substances in drinking and environmental water is essential for protecting human health and maintaining good environmental quality standards. Making accurate measurements at ultra- low levels is an essential part of any monitoring program. As more and more pesticides, pharmaceuticals and personal care products (PPPCP) are released into the environment, there is a growing demand for analytical methods with multiple compound groups.

The purpose of this work was to demonstrate a direct injection UPLC-MS/MS method for the ultra-low level determination pesticides, pharmaceuticals and personal care products in drinking and environmental waters. The method performance study was completed on an ACQUITY Premier™ LC System with a Xevo TQ Absolute™ mass spectrometry system.

A method validation study was carried out on drinking water and 2 environmental water matrices. The method performance was assessed using 3 spike levels at 10, 20, and 100 ng/L for all analytes, with 6 replicates at each level. Average method performance for trueness, repeatability, linearity, and sensitivity was assessed through inter and intra-laboratory studies.

Using direct injection on Xevo TQ Absolute™ system removes sample preparation and enables a simple, high-throughput analysis of PPPCP in drinking and environmental waters.

### 3.13.P-We129 Monitoring of Pharmaceutically Active Compounds in River Water from Tagus River Basin (Spain)

**Silvia Royano**, Adrián de la Torre, Irene Navarro and María de los Ángeles Martínez, CIEMAT, Spain

Pharmaceutically active compounds (PhACs) are molecules designed to interact with human or animal receptors but if they are present in the environment is because of their excessive use. More specifically, PhACs are mainly introduced in surface waters through wastewater discharges. Their presence in water can result in public health or environmental problems like antibiotic-resistant bacteria or endocrine disruptions and, consequently, some of these PhACs have been included in the Surface Water Watch List by the European Commission.

To address this issue, 77 river water samples were collected from 13 sampling points located throughout the Tagus river basin between 2020 and 2022. Then, the presence of 23 analytes belonging to several groups of PhACs as antibiotics,  $\beta$ -blocker agents, psychiatric drugs, analgesics, anti-inflammatories, lipids regulators, fungicides and anthelmintics were determined. Tagus River is the longest river of the Iberian Peninsula (1007 km, 80600 km<sup>2</sup> basin) and travels in an east-west direction, from the Central Spanish Plateau to Lisbon (Portugal) with an average annual flow of 456 m<sup>3</sup>/s.

Filtered samples (1L) were spiked with deuterated internal standards and extracted by solid phase extraction using Oasis HLB (500 mg, 6mL) cartridges and eluted with MeOH. Instrumental analysis was carried out with a UHPLC-MS/MS system.

Chromatographic separation was made by an ExionLC system coupled to a Triple Quad™ 3500 MS/MS system for the MS/MS analysis. MRM mode was performed to acquire two transitions per target analyte. The method was validated and method limits of quantifications ranged between 5 and 25 ng/L. Recovery for deuterated standards were always between 70 and 120%.

Excluding miconazole (<MLOQ), all the analytes were quantified in water samples. The antidepressant desvenlafaxine dominated PhAC levels (10.9  $\mu$ g/L mean and 100% quantification frequency, Qf) followed by the antibiotic erythromycin anhydrous (3.2  $\mu$ g/L mean and 82% Qf) and  $\beta$ -blockers irbesartan (745 ng/L mean and 82% Qf) and valsartan (526 ng/L mean and 88% Qf).

Sampling points located close to highly populated areas showed higher water PhAC concentrations.

### 3.13.P-We130 Antibiotics and Emerging Pathogens in the Ebro Delta, and the Albufera of Valencia

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Urban rivers are impacted ecosystems which may play an important role as reservoirs for antibiotic-resistant (AR) bacteria. The primary goal of this work was to assess antibiotics, the prevalence of antibiotic resistance, antibiotic resistance genes (ARGs) and bacteria (ARBs) in two selected areas in Spain, the Ebro Delta, which is a sewage-polluted river, and the Albufera of Valencia, which is a freshwater lagoon on the Gulf of Valencia in eastern Spain.

Main antibiotics groups were assessed by ultra-performance liquid chromatography coupled with high-resolution mass spectrometry tandem mass spectrometry (UPLC-HRMS/MS). ARGs were detected using qPCR to assess aquatic habitats' emerging microbial contaminants vulnerability. *Escherichia coli* and ARB were grown in parallel by cultivation methods.

Despite the occurrence of antibiotics being detected in almost all the samples, the concentrations were ranging between the MLOQ and 3.7  $\mu$ g/L. The most detected groups were quinolones such as ciprofloxacin and ofloxacin, the macrolides antibiotics such as azithromycin, beta lactam antibiotics as carbapenems, and sulphamide antibiotics such as sulfamethoxazole, and trimethoprim. In case of bacteria, despite the microbial load decreases in effluent compared to influent wastewater (2-3 log<sub>10</sub> on average), the reductions between upstream and downstream wastewater do not comply with the latest European standards, Regulation (EU) 2020/741 of the European Parliament and of the Council on minimum requirements for water reuse.

To conclude, thanks to the possible determination of ARGs and ARBs in samples, this work will provide a methodology which can be used as an early indicator for the tracing of pathogens and ARGs. Moreover, fecal contamination (*E. coli* and microbiological indicators) has been found in surface waters, even in treated water sources.

### 3.13.P-We131 Understanding the Connectivity of Pharmaceutical Pollution in River Catchments

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The near ubiquitous presence of pharmaceutical compounds in environmental waters represents an emerging cause for concern, but gaps remain in our understanding of how human and veterinary pharmaceuticals enter and travel through river catchments. A more holistic approach is needed in order to develop effective management strategies that conform to the catchment-based approach, although this is complicated by the patchy nature of available monitoring data for river water and by the significant seasonal variation in concentrations which makes comparisons even within datasets tenuous. Here, an exploration of pharmaceutical concentrations across the Aire catchment in the UK aims to provide insight into how the underlying connectivity of the catchment system, conceptualized as a source-pathway-receptor model, may determine observed patterns of contamination. To account for temporal variations of inputs and flow, samples collected on two separate occasions (corresponding to low and high flow conditions, respectively) were used to create two spatial snapshots for contamination with nine representative compounds. The snapshots were then used to explore spatial patterns in the catchment and what factors – topographic, physico-chemical, or related to potential sources and pathways for pharmaceutical pollution – may influence them. For the first snapshot, conducted in low flow conditions, none of the locations had concentrations above the limit of detection for five of the nine target analytes (Atenolol, Diclofenac, Erythromycin, Iopromide and Sulphadiazine). Results for the detected compounds have emphasized the difference in spatial patterns based on use category: as opposed to the veterinary use compound (Cypermethrin), the human use compounds (Carbamazepine, Lidocaine and Sucralose) showed significant correlation to contributing area, as well as to population served by the wastewater treatment plants upstream of the sampling sites and corresponding estimates for amounts of prescribed active ingredient. Sucralose also produced strong correlations to Carbamazepine and Lidocaine, supporting its use as a proxy for contamination with human pharmaceuticals, alongside the more frequently cited Carbamazepine. Ultimately, this research will inform the development of a graph representation of the system, used to assess the relative contribution of different pathways as they connect to the river channel and to inform as to the best intervention points within the catchment.

### 3.13.P-We132 Ecotoxicological Impact of Environmental Pharmaceuticals and Their Mixtures

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Investigations on adverse outcomes of pharmaceuticals mixtures represent nowadays a research priority to characterize the risk for marine ecosystems. Indeed, an increasing number of studies have recently highlighted the simultaneous occurrence of anti-inflammatories, lipid-lowering agents, cardiovascular and psychiatric drugs in seawater, sediment and aquatic wildlife. Evidence of pharmaceuticals capability to interfere with biochemical and physiological processes in non-target species, revealed the urgent need to prioritize the environmental sustainability of the most widespread compounds and the development of comprehensive risk assessment procedures. In the present study, the ecotoxicological potential of 15 pharmaceuticals belonging to different therapeutic classes was investigated through a battery of bioassays which includes the algal growth inhibition of *Phaeodactylum tricorutum*, the embryotoxicity assay with *Paracentrotus lividus* and the bioluminescence test with *Aliivibrio fischeri*. Pharmaceuticals were dosed both alone and in mixtures, at two different environmentally realistic concentrations (1 µg/L and 10 µg/L). Overall results were finally elaborated through a quantitative Weight Of Evidence (WOE) approach, which assign to each bioassay specific weights and thresholds based on the measured endpoint and sensitivity of the tested species, to finally provide a cumulative level of hazard of the entire bioassays battery. Obtained results highlighted different sensitivity of tested species toward selected pharmaceuticals and mixtures, with major effects on *P. tricorutum* and *P. lividus*. Although the overall elaboration with the WOE model revealed limited acute toxicity of pharmaceuticals, higher level of hazard was measured for mixtures compared to single compounds. Noteworthy, in mixtures the higher toxicity was not related to an exposure dose or to the number of combined drugs, suggesting the need of future investigations to unravel mechanisms of interaction among these molecules. In conclusion, the impact of pharmaceuticals cannot be predicted only by the evaluation of acute toxicity, and our results suggest the importance to integrate acute toxicity data with bioaccumulation and long-term detrimental effects for a more comprehensive risk assessment of these emerging contaminants in marine ecosystems.

### 3.13.P-We133 Residues of Drugs Used to Treat Diseases of Affluence and their Effects on Zebrafish Embryos (*Danio rerio*)

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In recent years, the incidence of diseases of affluence, such as diabetes mellitus, cardiovascular diseases, high blood pressure, and high cholesterol has been constantly increasing. Due to the increased prescription and consumption of drugs used to treat these diseases, concentrations of their residues are rising in the aquatic environment alarmingly. Common technologies used in wastewater treatments plants are not able to remove pharmaceutical substances completely. Thus, the residues of still biologically active substances enter surface waters and can be potentially dangerous for non-target aquatic organisms. The aim of the study was to evaluate effects of commonly used pharmaceuticals metoprolol, enalapril (at the concentration 0.1; 10; 100; 1 000, and

100 000 µg/L) metformin (at the concentration 1; 10; 100; 1 000, and 100 000 µg/L) on the embryonic stages of zebrafish (*Danio rerio*) using the embryonic acute toxicity test according to OECD method No 236. After four days of exposure, our results show that tested drugs influenced hatching rate at all tested concentration, which suggests that these substances might have the potential to disrupt early development of fish. Moreover, heartbeat rate was found to be affected as well. qPCR analysis indicated significant changes in the expression of genes responsible for the early development of embryos.

### **3.13.P-We134 The Effect of Environmentally Relevant Concentrations of Diclofenac, Ciprofloxacin, and a Binary Mixture on *Mytilus edulis* Fertility Potential**

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It has been extensively reported that pharmaceutical compounds are present on a global scale in the aquatic environment. It is of great importance that we do not underestimate the potential environmental risk that these chemicals pose, despite the low concentrations detected in the environment. This is especially true when multiple pharmaceutical contaminants are combined. Both diclofenac and ciprofloxacin have been suggested to cause reproductive toxicity, although test concentrations are often higher than those measured in the aquatic environment. In addition, it is suggested that aquatic spermatozoa may be more sensitive to exposure of these chemicals than oocytes.

The present study investigates the effect of environmentally relevant concentrations (500, 1000 and 10000 ng/L) of diclofenac, ciprofloxacin and a binary mixture on the fertility potential and spermatozoa function of mussels (*Mytilus edulis*). To determine the effect on fertilizing potential, both *in vivo* and *in vitro* exposure are carried out and successful fertilization and normal larvae development assessed. Specifically, male gametes are investigated after a 60-minute *in vitro* exposure to the selected pharmaceuticals for presences of reactive oxygen species (ROS), intact mitochondria, viability and total motility, as their impairment predicts decrease reproduction and consequently an impact at population and ecosystem level.

In the *in vivo* experiment, the fertilization rate and larvae development are evaluated after the exposure of adult individuals to the same chemical used in the previous testing.

Results indicate that, after 60 minutes *in vitro* exposure to environmentally relevant concentrations of diclofenac, ciprofloxacin and a binary mixture of the two, no significant difference ( $p=0,988$ ) in the percentage of positive ROS spermatozoa was observed, as compared to control. Despite showing no significant effects on ROS, previously mentioned assays may further elude into the possible mechanism behind the effect of the pharmaceuticals on reproductive function in male mussels and whether environmentally relevant concentrations decrease fertilization potential in marine invertebrates, especially when chronically exposed to the pharmaceuticals.

### **3.13.P-We135 Effects of Pharmaceuticals and Personal Care Products (PPCPs) on Plants, Soil Invertebrates and Ecosystem Service Delivery**

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The occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in the environment has spurred a quest for knowledge of the sources, pathways, occurrence, fate, and ecotoxicological effects on the ecosystems. Moreover, a corresponding increase in the effects of PPCPs is anticipated in the future since the demand and use of PPCPs keeps rising for obvious reasons such as existing and emerging diseases (for instance Covid and its mutants), economic & population growth, deteriorating climatic condition and a host of others. This research is focused on a deliberate attempt to link the effects of PPCPs on the terrestrial ecosystem and the corresponding impacts on ecosystem service delivery.

This study is considering the effects of some of the most frequently occurring pharmaceuticals (Antibiotics, Anti-epileptics and Non-steroidal anti-inflammatory Drugs and Personal Care Product) on terrestrial plants (wheat, rye, lettuce) and soil invertebrates using earthworm as a model organism. It starts with a systematic review of existing literature and published databases on the subject matter. This would be followed by pot experiments and determination of the effects of these chemicals on the test organisms/plants.

In the meantime, preliminary findings following a systematic review of previous publications and ecotoxicological database have revealed that PPCPs may have an inhibitory or lethal effect on the biochemistry, physiology, population and yield of plants, soil microbiota, soil invertebrates as well as disrupting the ecosystem service delivery. Several gaps were identified in the on-going systematic review such as missing data on the effect of metabolites, chemical cocktails or transformation products of PPCPs in soils and a need to elucidate microbiotic functions and their significant effects on the terrestrial ecosystem. As this project is still ongoing, the results will be revealed at the completion of the study. An ecosystem service assessment will be also conducted based on the result gotten from the study.

### **3.13.P-We136 Ecotoxicological Impact of Gentamicin, Streptomycin and Ampicillin on Soil and Water Bioindicators**

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Antibiotics are a group of pharmaceuticals in widespread use worldwide. In recent decades, their detection in aquatic and terrestrial environments has generated the need to know their impacts on non-target organisms in these habitats. There are still many antibiotics whose impact on key terrestrial and aquatic organisms is unknown, so more research is needed to complete this knowledge and obtain conclusive results.

Three widely consumed antibiotics detected in water bodies and soils were chosen in this study: gentamicin, streptomycin, and ampicillin. To assess ecotoxicological effects, three key non-target organisms were analyzed over a wide range of concentrations: *Daphnia magna* for aquatic ecosystems, *Eisenia foetida* for terrestrial ecosystems, and *Allium cepa* for plant toxicity.

In the case of *D. magna*, the concentrations tested varied according to the sensitivity of the antibiotic to the organism. The LC<sub>50</sub> (24 hours) value was the lowest for streptomycin 772.368 µg/mL, somewhat higher for gentamicin (LC<sub>50</sub>=840.751) and high for ampicillin (LC<sub>50</sub>=4324.134 µg/mL).

For *E. foetida*, tested concentrations were 0, 1, 10, 100 and 1000 mg/kg. No toxicity effects were observed even at the highest concentration.

For *A. cepa*, tested concentrations were 0, 3, 30, 300 and 3000 µg/mL. For gentamicin and streptomycin, LC<sub>50</sub> values were 42.567 and 535,318 µg/mL, respectively. No toxicity was detected in the case of ampicillin.

Our results suggest that acute toxicity effects in the environment are unlikely, as it is difficult to reach concentrations that show toxicity in selected non-target organisms. However, the effects of long-term exposure to these drugs, as well as their synergies with other emerging contaminants also present in the environment, need further investigation.

### 3.13.P-We137 Changes in Soil Microbiome and Plant Metabolome Caused by Swine Wastewater Irrigation

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The environmental and agricultural impact of livestock wastewater irrigation has not been concluded. This study investigated the changes in soil microbiome caused by swine wastewater irrigation and the effects on crops.

Pak choi was planted in three farm areas, each irrigated with groundwater (GW group), a reasonable amount of swine wastewater (RS group), and an excessive amount of swine wastewater (ES group). Topsoil and pak choi were sampled in each group (both n = 54). Homogenised soil samples were processed with Bacterial DNA isolation and purification and sequenced with 300 bp paired-end reads. The metagenomic analysis was carried out using QIIME2 pipeline. Univariate analyses and permutational multivariate analysis of variance were used to compare the differences among groups. Linear discriminant analysis Effect Size (LEfSe) was used to find microbial biomarkers. The metabolites in soil and pak choi were acquired by high-resolution mass spectrometry (HRMS) and analysed chemometrically. Partial least squares discriminant analysis (PLS-DA) was employed to compare the metabolite profiles among irrigation groups. The metabolites with higher scores of variable importance in projection were identified.

Irrigation with swine wastewater impacted the bacterial structure of the soil microbiome, resulting in the elevation of certain bacteria. Beta diversity showed that bacterial compositions in the RS and ES groups differed significantly from the GW (all pairwise  $p < 0.01$ ). Compared to the GW, *Luteimonas arsenica*, an arsenic-tolerant bacterium, was enriched in the RS. In addition, *Novosphingobium arabidopsis* and *Chlorella sorokiniana*, both resistant to chemical contaminants, were abundant in the ES. These bacteria were found to survive under high nutrient salt and pharmaceutical contamination provided by wastewater. The metabolites in soil and pak choi differed among irrigation groups. Antibiotic substances, lincomycin, tiamulin, and 1-deoxynojirimycin, increased with the amount of swine wastewater, which may explain the microbiome findings in the soil. Significantly negative correlations ( $p < 0.05$ ) were observed between the amount of swine wastewater and the abundance of cysteinyl-valyl-leucine and 7-methylguanine in pak choi.

This study discovered the microbiome shift in the soil irrigated with swine wastewater, likely resulting from the input of nutrients and antibiotics. The functions of microbial biomarkers on crop metabolism will be elucidated.

### 3.13.P-We138 Resistance of Cyanobacterium *Microcystis aeruginosa* to Diclofenac Measured by Various Markers

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*Microcystis aeruginosa* is one of the unicellular cyanobacteria used in the ecotoxicology tests. It is cosmopolitan species with high adaptation abilities. The natural water contains the pollutants, which can cause the toxic effect to cyanobacteria and change in phytoplankton structure. The pharmaceuticals are among other new emerging pollutants. The ecotoxicology tests are often performed using green algae, but having in mind the increasing problem with blooms, there is a need to perform risk assessment of pharmaceutical using cyanobacteria. The diclofenac (DCF) is one of the priority pharmaceuticals with proven high stability in waters and negative impact on organisms. Furthermore, the presence of DCF was noted in waters around world. In this study we aimed to check how the DCF impact *M. aeruginosa* biology. The research was scheduled to test the several biomarkers and physiological markers. The basis of the ecotoxicological test was the OECD 201 protocol with modification. The DCF concentration was 100 mg/L. The flow cytometry, the spectrophotometry and HPLC-DAD techniques were adapted. At the end of test other markers were tested: chlorophyll a fluorescence, pigments (carotenoids and chlorophylls, phycobilins), peptides and toxins (in buffer extract of cells, quantification by LC-MS). Stability of DCF in test conditions was proven by the HPLC-DAD quantification. The main metabolites of DCF were not noted in water. The growing of the *M. aeruginosa* was not impacted by the DCF, thereby can be stated that this pharmaceutical is not toxic for tested cyanobacteria. Generally, the stable DCF concentration in water means that the penetration of this chemical into cell is limited. The main lipophilic pigments (carotenoids and chlorophylls) and the microcystins production was not changed by DCF. At the start of experiment (first hours) the photosynthetic processes were altered, but cyanobacteria acclimated to new conditions. Research shows the *M. aeruginosa* resistance to the DCF toxic effects, most probably due to repulsion of the negatively charged cell of cyanobacteria and anion of DCF. This makes this cyanobacterium win the interspecies competition in the environment impacted by DCF.

### 3.13.P-We139 Chronic Exposure to Diclofenac Produces Reproductive and Biochemical Effects on the Cladoceran *Daphnia curvirostris*

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Diclofenac is a non-steroidal anti-inflammatory drug reported in waterbodies in concentrations ranging from ng L<sup>-1</sup> to µg L<sup>-1</sup>. Diclofenac is discharged in domestic and hospital wastewaters and persists in effluents after conventional wastewater treatments, causing toxic effects in aquatic biota in polluted environments. In particular, the zooplankton community can be affected by polluting pharmaceuticals even at low concentrations. In this study, we quantified the chronic effects of diclofenac on the reproduction and metabolic biomarkers (proteins, lipids, and carbohydrates) of the cladoceran *Daphnia curvirostris*. The acute toxicity (48 h) of diclofenac was determined. In the chronic studies, *D. curvirostris* was exposed to the sub-lethal diclofenac concentrations corresponding to LC<sub>1</sub>, LC<sub>5</sub>, LC<sub>10</sub>, and LC<sub>20</sub> (10.3, 14.4, 17.2 and 21.3 mg L<sup>-1</sup>, respectively), during 21 d at 25°C, 16:08 photoperiod, feeding with 8x10<sup>5</sup> cells mL<sup>-1</sup> of the green microalgae *Pseudokirchneriella subcapitata*. Survival, accumulated progeny, and the number of clutches were quantified daily, and the proteins, carbohydrates, and lipids were determined at the end of the bioassays. The determined LC<sub>50</sub> for diclofenac was 32.29 mg L<sup>-1</sup> (limits 25.58 to 38.32 mg L<sup>-1</sup>). The chronic assays indicated that accumulated progeny was significantly reduced in all concentrations of diclofenac; however, age to first reproduction significantly increased in all the concentrations of the drug compared with the control. The number of clutches was significantly affected at concentrations of 10.3, 14.4, and 21.3 mg L<sup>-1</sup> of diclofenac. The neonates of *D. curvirostris* had increased protein content, but carbohydrates and lipids were significantly decreased in all concentrations of diclofenac; nevertheless, in the highest concentration of diclofenac, all the biomarkers content decreased significantly. The results indicated that diclofenac causes damage in *D. curvirostris*, warning about the toxic effects of pharmaceuticals on the aquatic biota.

### 3.13.P-We140 How Diclofenac Affects the Early Life Stages of *Danio rerio*

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The presence of pharmaceutical products in the aquatic environment has become a global issue of increasing environmental concern. Diclofenac is a non-steroidal anti-inflammatory drug used worldwide in human and veterinary medicine and has an estimated production of 940 tons per year; for this reason, it was identified as one of the most important pharmaceutical active compounds detected in aquatic environments in various countries. This study aimed to evaluate the effects of diclofenac on the early life stages of the zebrafish *Danio rerio*. Fish embryos were exposed to concentrations of 1, 2, 4, 6, 8 and 10 mg L<sup>-1</sup> of diclofenac following the protocol proposed by OECD Guideline 236 to evaluate toxic effects. Toxic effects were determined during 7 d at 25°C, 16:08 photoperiod. Effects on the early life stages were assessed daily up to 144 h post-fertilization. Lethal endpoints assessed were coagulation, no tail detachment, no somite formation, and no heartbeat. Sublethal endpoints to assess the effects of diclofenac on embryos included the presence of edema and curved tail. Results show that LC<sub>50</sub> for diclofenac on embryos of *D. rerio* was 6.27 mg L<sup>-1</sup> (limits 4.72 to 7.91 mg L<sup>-1</sup>). The hatching rate of zebrafish embryos decreased by 40, 51.6, and 80% at concentrations of 6, 8, and 10 mg L<sup>-1</sup> of diclofenac, respectively. Exposure to this drug produced lethal effects, such as coagulation, from 24 to 72 hpf. Sublethal effects such as edema and curved tailed were observed at all tested concentrations from 48 to 144 hpf, and the effects increased as the concentration of diclofenac increased. The obtained results indicated that diclofenac could be an environmentally toxic compound with the potential to provoke lethal and sublethal effects in the early life stages of *D. rerio*, so it is essential to establish safe levels of this kind of drug in the aquatic environment.

### 3.13.P-We141 Toxic Effects of Diclofenac on the Green Microalgae *Scenedesmus quadricauda*

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Worldwide 940 tons of diclofenac are produced and consumed each year. The presence of this pharmaceutical in waterbodies is a risk for the plankton community. Moreover, when the microalgae are affected, significant effects could be expected in all the trophic structures in aquatic ecosystems. This study aimed to evaluate the effect of diclofenac on population growth in the concentration of photosynthetic pigments and in the main biomolecule composition in *Scenedesmus quadricauda*. Bioassays were performed starting with 50,000 cells mL<sup>-1</sup> in PCG medium, with 0.01, 0.1, 1, 10, and 100 mg L<sup>-1</sup> diclofenac, incubating with constant aeration and continuous illumination at 25°C for 96 hours. The population growth of *S. quadricauda* was not significantly modified at any concentration of the pharmaceutical. However, an increased frequency in atypical cells was observed as the concentration of diclofenac increased. The concentration of chlorophylls *a* and *b* for the microalgae exposed to diclofenac was similar to that assessed in the control. Nonetheless, carotenoid content decreased significantly in all concentrations of diclofenac. The protein concentration in the microalgae increased by 271, 338 and 339 %, respectively, for the drug concentrations of 1, 10, and 100 mg L<sup>-1</sup>, while carbohydrates increased by 204 and 207% with 10 and 100 mg L<sup>-1</sup> of diclofenac. The lipids concentration was significantly reduced at all diclofenac concentrations compared with the control. Although the population growth was not reduced for diclofenac, the algae were affected in their morphology and the main macromolecule content, modifying their composition that could affect the development of filter-feeding zooplankton in waterbodies. The results confirm the vulnerability of primary producers to pharmaceuticals such as diclofenac and allow us to conclude that its discharge to aquatic ecosystems must be regulated to avoid ecological impacts.

### 3.13.P-We142 Effects of Naproxen, Ibuprofen, and Diclofenac on Cyanobacterium *Synechocystis Salina* with Relation to Salinity of Medium

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Cyanobacteria of the species *Synechocystis salina* are very widespread, living in different types of water bodies. The *S. salina* studied in this work was isolated from the Baltic Sea, which is heavily polluted by pharmaceuticals, among other pollutants. Pharmaceuticals are designated as emerging contaminants and their impact on the environment can be significant even at relatively low concentrations. The bioavailability of pharmaceuticals in salt water can differ from that in fresh water. Studies available in the literature indicate that microalgae can remove pharmaceuticals from water. However, most studies on the subject focus on green algae, and there are few papers on cyanobacteria. Cyanobacteria are mixotrophic organisms, so they can obtain energy through photosynthesis, but also from chemosynthesis, theoretically taking pharmaceutical residues as source of organic carbon. Therefore, the aim of the present study was to investigate the effects of several frequently used pharmaceuticals from the group of non-steroidal anti-inflammatory drugs - NSAIDs, i.e.: naproxen (NPX), ibuprofen (IBF) and diclofenac (DCF) on *S. salina* and the effect of salinity on the toxicity of the tested NSAIDs. In order to meet the growing problems of pharmaceutical contamination in water, another objective of this study was to investigate the potential of *S. salina* to degrade selected NSAIDs. In order to carry out this research, 96-h tests were conducted in which cyanobacteria were exposed to NSAIDs at concentrations of 0.1 - 100 mg L<sup>-1</sup>, in basal culture medium and in medium with addition of sea salt (35‰). The study showed that IBF, NPX and DCF did not affect the growth of *S. salina*. In contrast, high salinity inhibited the growth of *S. salina*, but after some time the culture adapted. Salinity had no effect on the toxicity of the tested NSAIDs, and the cyanobacteria showed no potential to degrade the tested pharmaceuticals. The cyanobacteria's resistance to selected pharmaceuticals, as well as its inability to degrade them, may be due to the mutual repulsion of NSAID molecules with *S. salina* cells also carrying a negative charge. The high content of inorganic forms of carbon in the culture medium may also reduce the potential for degradation of NSAIDs.

### **3.13.P-We143 Psychoactive Drugs Alter Behavior in Non-target Species – Coiling Movements as a Sensitive Endpoint For Neurotoxicity In Zebrafish (*Danio rerio*) Embryos**

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The permanent increase in prescription and consumption rates of pharmaceuticals in our society results in elevated concentrations in open water bodies. These contaminants, including antidepressants, are of special concern, since they are designed to be stable and act already at low doses. Psychoactive drugs interfere with neurotransmitter homeostasis in synaptic clefts and the brain and can, thus, manipulate neurobehavioral patterns. Given the high conservation of binding sites across vertebrate groups, fish are likely to also be affected by unintentional exposure to such psychoactive drugs. Since, at early stages of development, the nervous system is particularly vulnerable to external stressors such as chemicals, early life-stage exposure bears the risk of irreversible adverse effects in later stages of life. This, there is a need for sensitive and robust testing systems in early life-stages of fish to determine potential risk of neurotoxic compounds in non-target species.

As a non-protected vertebrate model, zebrafish embryos have also received increasing attention for testing of behavioral effects within developmental neurotoxicity. Spontaneous coiling of the tail is the earliest detectable motor behavior and can relatively easily be recorded. The purpose of this study was to compare the effects by different antidepressants on coiling activity of zebrafish embryos. Movements were recorded from 21 to 47 hours post fertilization and analyzed with respect to coil duration and frequency. A generalized additive model (GAM) was applied to describe the complex linear and non-linear relationships between coiling behavior and multiple factors including batch, time, light phases, and contaminant concentration. As a major advantage, this approach allows to distinguish between non-treatment related effects (random effects) resulting from natural variation within groups and replicates as well as fixed treatment effects of the chemical concentrations. Thus, time-dependent alterations in behavioral patterns by antidepressants can also be identified within a complex exposure setting characterized by intrinsic high variability.

### **3.13.P-We144 Physiological and Biochemical Alterations in Zebrafish Exposed to an Antineoplastic Drug**

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Antineoplastics agents (AAs) are applied in cancer therapy, and they can end up in environmental compartments through the hospital and domestic wastewater. They usually enter the wastewater partially transformed or even unchanged via human excretion. The growing use of AAs is an emerging issue in environmental safety, which represents a problem when regarding Environmental Risk Assessment approaches. They are considered micropollutants and one of the least studied groups of pharmaceuticals being inadequately or not regulated. They are mainly cytotoxic at low concentrations which may pose a threat to aquatic organisms. One of the most used antibiotic agents with antineoplastic properties used in cancer treatment are anthracyclines. Epirubicin (EPI) is a chemotherapeutic agent belonging to the anthracycline drug class that acts by suppressing the DNA and RNA synthesis. It is indicated for treating several cancers. Approximately 11% of EPI is excreted unmetabolized via urine within 24 h.

The zebrafish *Danio rerio* is a well-established freshwater model organism that is widely used in both eco- and toxicological assessment of numerous contaminants. Zebrafish is a model which allows the evaluation of different apical and cellular/molecular endpoints (e.g., lethality, developmental, biochemical, and genotoxic alterations, locomotor behaviour) which will help to holistically understand the toxicity induced by a given contaminant. Thus, the present study aimed to assess the physiological and biochemical alterations of Epirubicin HCl (EPI HCl) in *Danio rerio* eggs.

The exposure followed the OECD 236 FET protocol and different endpoints were daily observed and documented during 96 h, such as mortality, hatching success, and malformations (e.g., pericardial oedema, incomplete yolk sac absorption, and tail deformities). Also, biomarkers for neurotoxicity and oxidative-stress and DNA damage (comet assay) were assessed. The behaviour assay occurred at the end of 120 h of exposure to EPI HCl using the ZebraBox (Viewpoint, Lyon, France) to track the locomotory activity of the larvae.

Results from the present study showed physiological, biochemical and genotoxic alterations at concentrations higher than the ones observed in wastewater effluents. Nonetheless, by integrating the endpoints at different levels of biological organization, we can establish adverse pathways and provide sound insight for regulatory proposes.

### 3.13.P-We145 Prediction of the Joint Toxicity of Anticancer Agents to the Zebrafish *Danio rerio*

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Pharmaceuticals are considered emerging contaminants and concerns about their environmental impact have been raised in the last decades as they have been detected in the environment. One group of pharmaceuticals commonly found in the environment are the anticancer agents (AAs). AAs are one of the least studied groups with respect to environmental hazard and may present specific risks to aquatic species. Under a real scenario, AAs (as other substances) do not exist alone in the environment but rather in mixtures with other AAs and/or chemical substances simultaneously. It is thus of urgent need to assess the effects of eventual mixture exposures to non-target aquatic species.

Aiming at understanding the potential hazard of AAs mixtures, the single and joint effects of three AAs (the alkaloid Trabectedin, the anthracycline Doxorubicin and the platinum-based agent Oxaliplatin) were evaluated in zebrafish embryos using the OECD 236 Fish Embryo Acute Toxicity (FET) Test Briefly, fertilized eggs were exposed initially to a series of concentrations of each AA for 96h. Once the LC<sub>50</sub> values were determined for each AA, they were transformed into Toxic Units (TU) and a full factorial design was used to predict their joint toxicity pattern. Additionally, the comet assay was performed using no-effect concentrations in order to evaluate DNA damage after 96h of exposure of embryos to the compounds. Embryos were exposed during 96h to a series of combinations of the three AAs (Trabectedin+Doxorubicin, Trabectedin+Oxaliplatin, and Doxorubicin+Oxaliplatin) along with control treatments. The MIXTOX model was used to predict their joint toxicity using as starting point the reference models for Concentration Addition and Independent Action.

The single acute toxicity tests showed that trabectedin, doxorubicin and oxaliplatin caused mortality and several malformations in zebrafish embryos/larvae, such as pericardial edema, yolk sac absorption and tail deformities, with values ranging from µg/L to mg/L. DNA damage was also promoted to larvae exposed to all three AAs after 96h at concentrations where no apical effects were observed. Preliminary results of the mixture exposure indicate interaction between the tested AAs.

### 3.13.P-We146 Isoxazoline Parasiticides: Should We Worry About Their Environmental Impact?

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Isoxazoline ectoparasiticides are a novel class of veterinary drugs. They are currently widely used thanks to their properties (broad spectrum, prolonged action, simple use).

Currently, these drugs are approved for use in dogs and cats but also used in poultry. Pharmacokinetic parameters indicate that these compounds are eliminated primarily in feces (90%) and have long half-lives in animals (5-13 days). In northernly carnivore species such as wild carnivores in captivity, external parasites can have deleterious effects and carry other pathogens. Therefore, zoo veterinarians are interested in using these effective and safe drugs. The purpose of our study was to determine some PK parameters in wild carnivore species (by means of fecal elimination) as well as in companion animals and to evaluate species differences as well as difference among the 4 different isoxazoline ectoparasiticides.

Our results indicate that some species like lions or mountain lions can eliminate significant residues of fluralaner in their feces for up to three months. Data regarding dogs and cats are still being analyzed.

Using published information on the susceptibility of insects, we conclude that dung beetle feeding on contaminated feces may be exposed to high (and potentially lethal) concentrations of isoxazoline ectoparasiticides for several weeks.

We suggest that Environmental Risk Assessment should be performed for these drugs, even though they are only used in companion animals and that the development of scoriifier scenarios could help cover this approach. In the meantime, it is strongly recommended to pet owners to collect their animals' feces and eliminate them in the garbage, especially if wastes are incinerated, to avoid any environmental diffusion of these compounds.

### 3.13.P-We147 Overview on the Diversity of Ectoparasiticide Veterinary Medicines Authorised for Cats and Dogs in the EU/EEA and the Related Insecticidal and Acaricidal Active Substances

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Current research on the presence of ectoparasiticide active substances used in veterinary medicinal products (VMPs) for companion animals in the environment predominantly focuses on the two active substances fipronil and imidacloprid. The actual



range of substances authorised in such VMPs, however, is much broader. The exposure pathways of these substances into the environment and their environmental concentrations are strongly determined by characteristics of the medicinal products such as the posology, the method of administration (e.g. spot-on, collar, tablet, spray, injection) as well as the mode of action (systemically or locally acting). Knowledge on the additional uses of some active substances for instance as biocide or pesticide ("multi-use substances") represents a hurdle in the interpretation of environmental concentrations. Such details are usually difficult to access for environmental researchers.

To provide an overview on the available knowledge on the types of ectoparasiticide VMPs currently authorised for cats and dogs in the EU/EEA and on the specific active substances that are included in these products, a survey was carried out in 2021 among the Member States to retrieve information on these products from databases of the national competent authorities. Together with information from further databases on VMPs currently authorised via national, decentralised and centralised procedures, a dataset of more than 1200 ectoparasiticide VMPs was obtained and evaluated. The evaluation showed that about 40 different ectoparasiticide and endectocidal active substances are included in VMPs authorised for cats and dogs in the EU/EEA. In addition, a rough estimate of the extent of their use across the Member States, as well as information on the approval status of these active substances within the EU biocidal and pesticidal legal frameworks are given.

This overview provides environmental researchers, regulators, and managers with a knowledge base on ectoparasiticide medicinal products currently authorised in the EU/EEA for cats and dogs and their active substances. It also provides a status quo of these substances' uses in the different regulatory frameworks, which is essential for exposure modelling and interpretation of environmental concentrations and for planning future studies.

### **3.13.P-We148 Imidacloprid in SWTP and Watercourses in Spain: Are Veterinary Medicines a Significant Source of Emission?**

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Imidacloprid is a commonly used neonicotinoid insecticide that has been authorised as plant protection product (PPP), as biocide (BP) and as Veterinary Medicinal Product (VMP). However, strict regulations have been established in the European Union (EU) during the last two decades to control the concentration levels of imidacloprid (among other water contaminants) in the water environment. In Spain, monitoring of imidacloprid by following the indications reflected in the Water Framework Directive (WFD) have been performed since 2018 and have been extended until (at least) 2021. Within this framework, samples in Sewage Water Treatment Plants (SWTP) and watercourses were taken.

The main aim of this work is to summarize the results obtained from the monitoring of imidacloprid in Spain during the years 2018 and 2019. Furthermore, a reflection of the possible sources of emission of imidacloprid residues is performed. Quantifiable levels of imidacloprid, that might suppose a risk for the aquatic environment, were detected in almost all SWTP and watercourses sampled in Spain in 2018 and 2019. Further water samples have been taken during the years 2020 and 2021 although the results are not available yet. It seems that PPP and VMPs might be the main sources of imidacloprid to the aquatic environment. However, as the uses of imidacloprid as PPP are no longer authorised, special attention should be paid to the residues detected in the following years to obtain a more reliable conclusion on which could be the main imidacloprid sources.

### **3.13.P-We149 Fur Used in Nest Building by Birds Potentially Contributes to Pesticide Exposure**

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The fate and environmental impact of insecticides used as ectoparasitic treatment for companion animals is obscure. It has long been assumed that veterinary medicinal products used in pets are likely to be associated with fewer environmental concerns than those in food producing animals. However, there are an estimated 9.9 million dogs and 10.9 million cats in the UK alone (PDSA, 2019), and approximately 80% of dogs and 82% of cats are treated for fleas. Many cat and dog flea treatments contain either fipronil or imidacloprid, which have been banned from agricultural use since 2017 and 2018 respectively. Treatment is conducted as often as monthly and will remain on fur for a long period of time as they are mainly applied topically in the form of shampoos, spot-on, sprays, dips, foams, collars or even powders. We collected fur from the nest lining of 103 nests from blue tit (*Cyanistes caeruleus*) and great tit (*Parus major*) collected by volunteers both from rural (62% of nests) and urban (38%) habitat. Using Liquid Chromatography Mass Spectrometry methods, we screened the nests for 24 different pesticide compounds. Eighteen pesticides were detected, with the number of compounds found per nest ranging from 2 to 12. The insecticides fipronil, imidacloprid and permethrin were detected in 100%, 88.3% and 87.3% of samples, respectively. Across nest samples, the mean concentrations were: fipronil (115 ppb), imidacloprid (360 ppb) and permethrin (208 ppb). Additionally, the highest concentration found was for dinotefuran, in the single sample where it was detected, at 7,198 part per billion (ppb). We found that the concentration of clothianidin, imidacloprid, thiacloprid, and fipronil was positively linked to either chick survival and/or egg hatching rate, while permethrin and cypermethrin were negatively correlated. These findings suggest that dermal contamination and ingestion through preening behaviour from the nestlings could be a route of exposure. We argue for a re-evaluation of the environmental risk associated with dermal exposure of chicks to commonly used pesticides and parasiticides.

### **3.13.P-We150 Pet Dogs Transfer Veterinary Medicines to the Environment**

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Worldwide, the number of pet dogs increases yearly, and as a result so does the use of veterinary medicines for flea and tick control. We investigated the potential transfer of veterinary flea products from dogs to the environment in a 'proof of principle' experiment. For this purpose, samples of hair, urine, and water after swimming were investigated. Nine dogs were recruited for this study, eight of which had been recently treated with an ectoparasiticide product. Hair and urine samples were tested for afoxolaner, fluralaner, fipronil and imidacloprid. Interestingly, contamination with ectoparasiticides was frequently demonstrated in samples from dogs untreated with these particular substances, suggesting widespread secondary transfer. In addition, hair retrieved from a bird's nest contained fipronil, fluralaner and imidacloprid, indicating a potential pathway for the exposure of juvenile birds. Three of the dogs also participated in a swimming experiment. One had been treated with oral fluralaner, whilst the remaining two had received other compounds not included in our study. However, in all three dogs, both fluralaner and imidacloprid were detected in hair samples. Fluralaner concentrations in the swimming water exceeded Dutch water quality standards, indicating a potential risk to the aquatic environment. Imidacloprid levels increased after each swimming dog but did not breach Dutch water quality standard levels. These findings all call for improvements in the current risk assessment and management for veterinary medicines, by including companion animals and their exposure pathways into ecosystems.

### 3.13.P-We151 The Consequences of Misinformation: Acute Effects of a Mixture Between Polystyrene Nanoplastic and Ivermectin on Invertebrates and Green Algae

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Environmental pollution is becoming increasingly complex due to the number of compounds being discharged into the water bodies. Rivers will carry plastic and chemical pollution toward the ocean or lakes making plastic a pollutant that is considered ubiquitous in the environment even in regions without human occupation. In the environment species are concomitantly exposed to several chemicals such as medicines and plastics. Among the most used veterinary antiparasitic drugs is ivermectin (IVM), which has seen a surge in consumption due to its supposed effect on avoiding COVID-19 infections but has already been proven false. IVM is mostly excreted through feces, it can be present in the environment in concentrations that pose a risk to the biota. *Daphnia magna* is known for its sensibility to IVM, while the effects on algae are yet unknown. This work aims to help bridge the gap between laboratory and environmental conditions by testing the mixture between polystyrene nanoplastics (PSNP) and IVM. Both compounds are hydrophobic thus indicating a possible chemical interaction, that could alter the effect of the mixture. Acute toxicity tests were performed with *Daphnia magna*, following OECD 202 resulting in an EC50 of  $126.4 \pm 17.4$  mg/L for the PSNP and  $7.63 \pm 17.4$  ng/L for the IVM. While the algae growth inhibition was performed following the *Chlorella vulgaris*, *Raphidocelis subcapitata*, and *Chlamydomonas reinhardtii*. Their mixture was evaluated following the Abbott method to indicate whether the mixture was synergetic or antagonistic. A brief risk assessment of the mixture was also performed. The results from this evaluation can help regulatory bodies understand if their water quality standards are being too permissive by not including these emerging contaminants in their legislations. When including contaminants such as IVM, will help them verify if the established concentration is safe enough given the mixture scenario in the environment.

### 3.13.P-We152 Biological Effects of Simultaneous and Separated Exposure of Mussels to Citalopram/Bezafibrate and Polyethylene Microplastics in Seawater

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Pharmaceutical active compounds (PhACs) have been detected in the different marine environmental compartments and their occurrence in marine organisms raises concerns regarding toxic effects. Similarly, it is still little known the role of microplastics (MPs) as pollutant vectors in the marine environment, particularly for contaminants of emerging concern such as PhACs. MPs can sorb pollutants in their surface, including PhACs, so MPs could modulate the bioavailability of PhACs for aquatic organisms, impacting their associated effects. Mussels have been proposed as model organism in ecotoxicology studies, representing suitable bioindicators for environmental monitoring and toxicity test. In this study, the aim was to evaluate the effects of polyethylene MPs and selected PhACs, considering separated exposures of mussels (*Mytilus galloprovincialis*) to the antidepressant citalopram (CIT), the lipid regulator bezafibrate (BEZ), and polyethylene microplastics (MPs 4-6 µm (PE)), and the corresponding PE-PhAC mixtures, besides a control without contaminants. Individuals were sampled twice during exposure (10 and 21 days), and after depuration (28 days), to obtain the digestive glands and gills tissues for biomarker analyses. Mussel's gills were used for oxidative stress analysis (lipid peroxidation, acetyl cholinesterase, glutathione peroxidase, glutathione S-transferase and catalase activity). The analyses carried out in mussel gills showed results with greater sensitivity than those obtained in the digestive glands. Mussels accumulated PhACs over the experiment, with higher concentrations of CIT than BEZ, maintaining relatively constant levels during the exposure period (mean: 763 ng/g dw for CIT and 2 ng/g dw for BEZ). In the depuration period, a partial release of CIT, previously accumulated in the organisms, was observed in seawater and mussel tissue. The results suggest that PhACs can be significantly accumulated in mussels, especially CIT, and that they can be removed from organisms when the exposure to the PhACs ceases. On the other hand, it was observed in a first period of 10 days, the mussels increased their antioxidant activity in those treatments with PhACs, appearing increases in cell damage at 21 days exposure. After the exposure

to environmentally representative concentrations, it can be observed how the treatments that have contained MPs tend to have higher levels of cell damage than those that have PhACs dissolved.

### 3.13.P-We153 Bioaccumulation and Biological Effects of Triclosan, Polyethylene, and Polystyrene Microplastics on Mussels (*Mytilus galloprovincialis*) in Seawater

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Personal care products have been detected in the different marine environmental compartments and their occurrence in marine organisms raises concerns regarding toxic effects. Triclosan (TCS) is an antibacterial agent widely present in consumer products. TCS has a highly hydrophobic nature with high log K<sub>ow</sub> value as 4.76, that it is likely to be sorb in sediments and other particles present in the surrounding environment, such as microplastics (MPs). MPs can sorb pollutants on their surface, impacting their bioavailability and associated effects. The bioaccumulation and the adverse effects caused by the separate and co-exposure of microplastics (PE and PS) and TCS on mussels (*Mytilus galloprovincialis*) in seawater were evaluated. An initial period of exposure (3 days) of mussels to high concentrations of TCS was applied, followed by 10 days of low environmental realistic concentrations and 7 days of depuration period to assess their resilience capacity. Bioaccumulation in soft tissues was determined using QuEChERS extraction followed by stir bar sorptive extraction coupled to gas chromatography with mass spectrometry. Oxidative stress and neurotoxicity (acetylcholinesterase, lipid peroxidation, catalase activity, glutathione-S-transferase and glutathione reductase) were analyzed in gills. Total Oxidant Scavenging Capacity Assay (TOSCA) toward peroxy and hydroxyl radicals using gas-chromatographic were analyzed in digestive gland. The accumulation of MPs in the digestive gland of the organisms was also analyzed. The results obtained showed similar bioaccumulation of TCS in mussels at the highest concentrations of exposure with and without MPs, producing an oxidative stress response on mussel tissues. The concentration of TCS was reduced in all cases when the exposure concentration decreased, specially after the depuration period, but the contaminant was still detectable. The detection of methyl-TCS, an anaerobic degradation intermediate of TCS, in mussel tissues was also relevant.

### 3.13.P-We154 Biological Responses in Digestive Gland of Mussels (*Mytilus galloprovincialis*) Exposed to Citalopram/Bezafibrate and Polyethylene Microplastics in Seawater

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Pharmaceuticals (PhACs) have been detected at low concentrations (ng/L) in the marine environment, particularly in coastal areas and in several compartments, including biota. PhACs are very often found together with other contaminants of emerging concern, such as microplastics (MPs). MPs can sorb pollutants on their surface, impacting their bioavailability and associated effects. Antidepressants and lipid regulators, such as citalopram (CIT) and bezafibrate (BEZ), constitute good examples of relatively bioaccumulative PhAC classes (log K<sub>ow</sub> > 3). The bioaccumulation and the adverse effects caused by the separate and co-exposure of microplastics (PE) and pharmaceuticals (PhACs) on mussels (*Mytilus galloprovincialis*) in seawater were evaluated. Mussels were exposed to polyethylene MPs 4-6 µm (PE), BEZ and CIT, and the corresponding PE-PhAC mixtures, besides a control without contaminants. Individuals were collected at 10 and 21 days followed by a depuration period of 7 days. Mussel's digestive glands were used for oxidative stress analyses: Total Oxidant Scavenging Capacity Assay (TOSCA) toward peroxy and hydroxyl radicals using gas-chromatographic and gene expression analysis (*Cat*, *Se-GPx*, *SOD*, *Acox*, *GST-pi*, *abcb1*) using a Real time PCR. The results showed an increase in the *GST-pi* gene expression after 10 days of exposure for different treatments respect to the control, while *Se-GPx* gene expression showed an increasing trend for treatments containing BZ as well as a decreasing trend for TOSCA, in contrast to treatments containing CIT. Thus PE coexposure did not affect to the effects of the PhACs.

### 3.13.P-We155 Comparison of Environmental Impact of Five Pain-Relief Medicines

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In the Netherlands, there is a large market for self-care products, with a yearly revenue of over 800 million euros. A large share of this market consists of pain-relief (analgesic) medicines. Self-care pain-relief medicines like paracetamol and the NSAIDs aspirin, diclofenac, ibuprofen and naproxen of course benefit the user but may also impact the environment. Known negative effects of NSAIDs are tissue damage in aquatic organisms and secondary poisoning. Because of these concerns, diclofenac and ibuprofen are proposed as priority substances within the Water Framework Directive. From various stakeholders in the health care sector questions have been raised whether the impact of pain-relief medicines on the environment differs between compounds and which of these could be chosen if environmental impact is taken into account besides patient benefits and side effects. Therefore the Dutch National Institute of Public Health and the Environment was asked to compare the environmental impact of aspirin, diclofenac, ibuprofen, naproxen and paracetamol. This poster presents the preliminary results of this study, including a risk assessment based on recent monitoring data.

### 3.13.P-We156 Defining the Data Gap: What Do We Know About the Exposure and Risks of Pharmaceuticals into The European Aquatic Environment?

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Identifying the potential risks from pharmaceutical residues in the environment is a goal and current strategic direction of environmental management described in the EU Strategic Approach to Pharmaceuticals in the Environment and the Green Deal. This is complicated by a paucity of high-quality monitoring and toxicity data to adequately describe risks posed to the riverine ecosystems by human and veterinary medicines - critical data gaps in the knowledge base needed to inform policy. The European Medicines Agency (EMA) advocates models in its environmental risk assessment (ERA) guidelines to fill data gaps, although the accuracy and efficacy of such methods vary.

In this study we analyze measured environmental concentrations (MECs) at 5933 sites in 25 European countries from EMPODAT and UBA monitoring program data from 1997 to 2020, and experimentally-derived toxicity data from the U.S. EPA ECOTOX database. Although 1764 uniquely identifiable active pharmaceutical ingredients are registered with the EMA for sale in the European Economic Area (EEA), only 312 (17.7%) of these are included in publicly available monitoring data, and only 36 (1.8%) compounds have sufficient ecotoxicological data to perform an EMA-compliant ERA.

Among the 27 compounds with sufficient empirical exposure and hazard data to conduct a single substance risk assessment according to EMA guidelines, four compounds (14.8%) had a median risk quotient (RQ) > 1. Endocrine disruptors have the highest median RQ, with 7.0 and 5.6 for 17 $\alpha$ -ethinyl-estradiol and 17 $\beta$ -estradiol respectively. A comparison of modelled exposures vs empirical data for 72 APIs demonstrated the high level of protectiveness of the current EMA guidelines, showing predicted environmental concentrations (PECs) exceeding median MECs in 98.6% of cases, with a 100-fold median increase. A total of 70 (97.2%) of compounds exceeded the 10ng/L PEC threshold for phase 1 EMA risk assessment using *in-silico* methods, compared to 40 (55.6%) using median values from empirical monitoring data.

This study describes and summarizes the data shortfalls hindering an accurate assessment of the risk posed to European waterways by pharmaceuticals, and identifies APIs about which neither their ecotoxicity or occurrence is known. Mandatory, central reporting requirements for monitoring data, and targeted ecotoxicity testing may alleviate the paucity of empirical data available for effective and accurate characterization of API risks.

### 3.13.P-We157 An Updated Concordance Assessment of Predicted-No-Effect-Concentration (PNECs) Aquatic Toxicity Data for Pharmaceuticals

**Tim Verslycke** and **Ifeoluwa Bamgbose**, *Gradient, Boston*

Environmental safety assessments are required for market approval of new Active Pharmaceutical Ingredients (APIs), however, such assessments are lacking for older drugs. To determine whether existing environmental toxicity data can be used to estimate predicted-no-effect-concentrations (PNECs) for APIs for which effect data is lacking, we analyzed publically available data from 197 APIs and presented our findings at the SETAC 2012 Berlin Meeting (Verslycke T, Mastrocco F, Lemay JC. 2012. poster number TU068).

Here, we present an update to our previous assessment by incorporating new PNECs that have been generated over the last decade and reported into the Swedish National Formulary (FASS.se) database. Following an outlier analysis, the updated dataset was used to evaluate statistical correlations between the PNEC and trophic level (algae, fish, and invertebrate), benchmark type (EC<sub>50</sub>/NOEC), assessment factor, and Anatomical Therapeutic Chemical (ATC) Classification System group. Based on the findings of the statistical analyses, two potential approaches are presented for developing PNECs for APIs for which no effect data are available along with their strengths and weaknesses.

### 3.13.P-We158 Do New Generations of Active Pharmaceuticals for Human Use Require an Adaption of the Environmental Risk Assessment? Part II: Case Studies

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In 2006, the European Medicines Agency (EMA) adopted a guideline on the environmental risk assessment (ERA) of medicinal products for human use. Since then, a large number of active pharmaceutical ingredients (APIs) with specific Mode-of-Actions (MoAs) that are potentially effective in the aquatic environment in concentrations lower than 0.01  $\mu$ g/L have been approved. This raised concern that the guideline does not allow a sufficiently protective environmental risk assessment for all of these new and very specific APIs and adaptations might be necessary.

A test strategy proposed after a previously conducted literature research should enable identification of effects specifically related to the MoA of the API and/or effects which occur at concentrations lower than the endpoints derived in the current standard long-term toxicity test set on fish, daphnia and algae. During the literature research, a number of test systems with potentially high sensitivity to these new APIs were identified. These tests included the *Lemna sp.* Growth Inhibition Test (OECD 221), the *Danio rerio* Fish Embryo Test (OECD 236) amended with sublethal endpoints, and the comet assay with environmentally relevant cell types derived from *Daphnia magna* and *D. rerio*.

Following 18 substances of the group oncologicals or cardiologicals and statins were assayed in these tests. The data generated according to the proposed new test strategy was compared to data from European public assessment reports (EPARs) to evaluate the level of protectiveness.

It was observed that the *Lemna sp.* growth inhibition test (OECD 221) is a suitable test system to be integrated into the environmental risk assessment (ERA) of specific classes of human pharmaceuticals (e.g. statins), while the other two short-term test systems (fish embryo toxicity test amended with sub-lethal endpoints and the comet assay) were in none of the cases

examined more sensitive than the currently employed apical endpoints from chronic aquatic toxicity tests (OECD 201, 211, 210) and can at best provide additional information. The test with *Lemna sp.* was more sensitive than the standard endpoints in the EPARs for one substance with a pharmacological MoA relating to the mevalonate pathway and to two tyrosine kinase inhibitors. Thus, the *Lemna* growth inhibition test might be a relevant additional test for the ERA of at least some APIs.

### **3.13.P-We159 Establishment of a Novel Public Database and Digital Assessment System (DAS) on Pharmaceuticals in the Environment (PIE) in the EU**

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A precondition for data-driven prioritization of active pharmaceutical ingredients (APIs) in the environment (PIE) is the availability and accessibility of relevant and reliable data.

However, today there is no database in the EU that could be used to inform data-driven prioritization or environmental risk assessments (ERA) of APIs. While relevant and reliable environmental data exists for many APIs, as ERAs are formally required as part of the marketing authorization procedure for medicinal products since 2006, these datasets are not consistently made public, findable by stakeholders, or organized in a systematic way. At the same time, there is growing interest from stakeholders to increase accessibility and transparency on available environmental data for APIs. For many of these issues, consolidation of all ERA data at one site would be a solution. It would also allow identification and prioritization of APIs with data gaps.

The public private partnership IMI PREMIER project ([www.imi-premier.eu](http://www.imi-premier.eu)) is a consortium of academia, authorities, SMEs, and pharmaceutical industry partners, which aims to provide a solution and to establish a database on environmental data for APIs in the EU. This database will be provided together with a novel publicly accessible digital assessment system (DAS), which can be used by stakeholders to perform risk assessments for pharmaceuticals.

The database in the DAS will be populated by experimental fate and ecotoxicological data generated for marketing authorization and provided by the EFPIA industry partners in PREMIER. Furthermore, there will be data from other databases and literature. The DAS aims to provide a high level of details on the experimental data. Additional elements such as API identifiers will be provided. There will be tools to search the database, algorithms developed within the PREMIER project to assess and evaluate the data presented, and guidance. Stakeholders shall be enabled to identify and collate all the information they need for their purposes.

Sustainability of the DAS is a key goal in the PREMIER project and the plan is that it will be made accessible and navigable by stakeholders for free. Our ambition is that the DAS will be hosted and maintained beyond the PREMIER project by European authorities.

### **3.13.P-We160 The ZERDA Database – A Collection of Data from the Environmental Risk Assessment of Pharmaceuticals**

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Since the mid-2000s, there have been EU-wide legal assessment concepts that allow a detailed and standardized investigation of the behavior and effects of active pharmaceutical ingredients (APIs) used in human and veterinary medicinal products (HMP/VMP) in the environment.

The results of these environmental risk assessments (ERAs) are partly published as endpoints (e.g. the adsorption coefficient normalized to the organic carbon content ( $K_{oc}$ ), the half-life ( $DT_{50}$ ), or the no observable effect concentration (NOEC)) in (European) Public Assessment Reports (EPARs/PARs) and are then, in contrast to the marketing authorization documents, in principle publicly accessible.

However, the EPARs/PARs are stored as PDF documents on sub-websites of various institutions and are therefore neither easy to find nor convenient to use. Up to now, no database exists in which all environmental information on APIs from EPARs/PARs is collected centrally.

The aim of this project was to collect all publicly available endpoints from ERAs of HMPs and VMPs marketed in Europe and to compile them in a database in a convenient and comprehensible way.

In the first step, all EPAR/PARs containing these data were compiled. For this purpose, a web crawler written in Python was developed to automatically find and download these reports. The endpoints were then extracted from the collected reports by another Python program and integrated into a database. Afterwards, a two-step quality assurance was performed.

This poster will show the detailed steps to harvest the (E)PAR documents and an overview on the ERA extraction results.

Furthermore, we will provide characteristics about the created database. Currently, the database contains endpoints for 463 APIs used in HMPs and 65 APIs used in VMPs. The extent is very heterogenic, from only predicted environmental concentration (PEC) values to comprehensive data for the compartments water and soil. This data collection allows us to assess the quality of EPARs with a view to ERA results and gives an interesting overview on the data availability from APIs marketed in HMP and VMP in Europe.

### **3.13.P-We161 Extended Environmental Risk Assessment for Sitagliptin**

*Lisa Ziv<sup>1</sup> and Joan G. Tell<sup>2</sup>, (1)Merck, (2)MSD*

This voluntary extended environmental risk assessment (eERA) considers the active pharmaceutical ingredient, sitagliptin, a

dipeptidyl peptidase 4 inhibitor indicated for treatment of type 2 diabetes mellitus. Sitagliptin was first approved in the United States in 2006 and has since been approved for use throughout most of the world.

The risk of an adverse environmental impact from use of sitagliptin was evaluated according to guidance provided by the United States Food and Drug Administration and by the European Medicines Agency. The results of the assessment concluded that sitagliptin is unlikely to pose a risk to the environment. Based on water solubility and log K<sub>ow</sub>, sitagliptin is not expected to bioaccumulate and is not considered to be a PBT (persistent, bioaccumulative, and toxic) compound. PEC/PNEC (predicted environmental concentration/predicted no effect concentration) ratios with respect to water, groundwater, sediment and microorganisms are all less than regulatory thresholds.

This eERa, presented herein, serves to share the available environmental test data compiled to date, while supporting our company's product stewardship commitments to continue to focus on identifying and preventing or minimizing potential safety and environmental hazards throughout the product's life cycle. Based on recently available measured concentration data in the literature, sitagliptin was found in river waters on all continents except Antarctica. However, based upon the low concentrations observed and ongoing expected usage, sitagliptin will continue to be unlikely to pose a risk to the environment.

### **3.13.P-We162 Diclofenac in Recycled Fertilisers: Screening Risk Assessment Identifies Low Risk but There Are Various Uncertainties and Need for Monitoring**

*Nele Deleebeeck, Michiel Claessens and Laura Lefèvre, Arcadis Belgium, Belgium*

The findings presented in this poster resulted from an assessment which was performed in view of a project for the European Commission, Directorate General Environment. In this project, the exposure and risk of contaminants/impurities of potential concern in fertilisers needed to be assessed, to identify contaminants/impurities or fertilisers for which additional regulatory measures may be required. Although various types of pharmaceuticals were considered eligible for risk assessment, diclofenac was the only pharmaceutical for which a case was set up within this project. The focus was on the potential risk from diclofenac in fertilisers based on/containing precipitated P salts derived from sewage sludge, and a screening level risk assessment was performed for the different environmental compartments, secondary poisoning, as well as humans exposed via the environment. Although there are indications of high removal efficiency of diclofenac during P salt precipitation, there is only limited experimental data available. Therefore, as a reasonable worst case, two scenarios with 1 and 10% transfer from sewage sludge to recycled material were included. Risk characterisation ratios (RCRs) were typically < 1 except for soil and secondary poisoning. However, when only considering the addition through the assessed use, RCRs would be below 1. Background concentrations (used as PEC<sub>regional</sub>) were close to or even above the PNEC values for the environmental compartments under consideration and therefore determined the outcome of the assessment rather than the contribution via recycled fertilisers. Source contribution analysis learned that important contributors to emissions of diclofenac to the agricultural environment are likely the use of raw or anaerobically digested sewage sludge and irrigation water. Manure might be an additional source for diclofenac input into the agricultural environment, however, monitoring data were lacking to confirm this. High local/regional differences are expected but could not be assessed in detail. Although low risk was identified in this screening assessment, attention will be drawn to the main sources of uncertainty in this assessment and their potential impact on the outcome of the assessment.

### **3.13.P-We163 UV-Light Absorbance of Natural Water Regulates the Bioavailability of Ciprofloxacin to Cyanobacteria**

*Qiyun Zhang, Ph.D. candidate<sup>1</sup>, Karel De Schampheleere<sup>1</sup> and Kristof Demeestere<sup>2</sup>, (1)Ghent University - GhEnToxLab, Belgium, (2)Ghent University, Belgium*

Recently, dissolved organic carbon (DOC) was found to regulate the ecotoxicity of fluoroquinolone antibiotic ciprofloxacin (CIP). Through the various interactions, CIP molecules bound to DOC increase in molecular size and polarity, which inhibits them from traversing the membrane system of aquatic organisms and decreases the observed ecotoxicity of CIP. As the brownification of many freshwater ecosystems is predicted to rise due to climate change, the importance of DOC on the bioavailability of CIP in nature might increase consequently. In this study, we investigated how DOC of different origins affects CIP bioavailability to cyanobacteria using a cyanobacteria toxicity test performed with *Microcystis aeruginosa* PCC7806. The bioavailability was expressed by the ecotoxicity of CIP observed in test media dissolved with different DOC compounds: (1) freshwater DOC in the naturally occurring states, collected from six rivers and lakes in Europe, (2) processed DOC extracted from the river Schwarzbach, and (3) commercial DOC product Suwannee River organic matter. The impact of DOC was quantified via describing the CIP<sup>+/</sup>-DOC interaction using a binding constant ( $K_{d,CIP^{+/}}$ ), which was related to the concentration of DOC and the ultraviolet (UV) light absorbance test media at 350 nm ( $A_{350}$ ). Observations showed that the composition of DOC clearly affected the ecotoxicity of CIP. When around 11 mg L<sup>-1</sup> different DOC compounds were present, the 50% effect concentration of CIP (EC<sub>50</sub>) varied by 10-fold, between 1.4 µg L<sup>-1</sup> and 15.2 µg L<sup>-1</sup>. Thus, DOC concentration alone is not sufficient to predict CIP bioavailability. Rather, the  $A_{350}$  of the test media demonstrated a significant positive correlation with the observed EC<sub>50</sub>, while the absorption coefficient ( $\epsilon_{350}$ ) correlated significantly with the  $K_{d,CIP^{+/}}$ . Our findings suggest that the light absorbance of natural water might be a better predictor for the impact of both processed and unprocessed DOC on CIP bioavailability.

### **3.13.P-We164 The Transmission Pathway of Multidrug Resistance Genes During Manure Application to Soil**

*Ewa Korzeniewska, University of Warmia and Mazury in Olsztyn, Poland*

Antibiotics are growing environmental contaminants leading to public health concern due to enhancing the occurrence of antibiotic resistance genes (ARGs). In the world, antibiotics are frequently used as the growth promoters and commonly as the therapeutic agents in poultry feed. Unfortunately, they are not completely metabolized in the body of chicken and ultimately excreted as poultry droppings. Therefore, usage of droppings as manure in agriculture may result in the promotion of co-occurrence of ARGs in the soil and ultimately leads to their transfer to crops and surface water via groundwater. In the present

study metagenome of poultry and bovine manure, soil and crops (parsley) samples was analysed for multidrug resistance (MDR) genes presence using deep sequencing method. Metagenomic analysis indicated that MDR genes were the most abundant in resistome of all analysed samples: manure, soil, groundwater, crops (parsley root and leaves). The obtained data indicate the transfer of MDR genes to the crops in both: the control (0.12 ppm) and manure-fertilised (0.68 ppm) soil samples. Therefore, the use of poultry and bovine manure as a fertilizer may carries the risk of inducing the spread of ARGs and, at the same time, the resistance phenomenon in agriculture and natural environments. Conducted research stands for another step in enhancing knowledge of possible dissemination pathways of resistant determinants in the agriculture environment.

### 3.13.V Human and Veterinary Pharmaceuticals in the Environment – Risk, Prioritisation & New Insights

#### 3.13.V-01 Microextraction and Chromatographic Determination of Diazepam and Clonazepam in Wastewater Samples *Silumko Nzube and Vernon S Somerset, Cape Peninsula University of Technology, South Africa*

Pharmaceutical pollutants entering the aquatic environment have become a growing environmental concern. These pharmaceuticals are unique pollutants because of their special characteristics and behaviour that cannot be simulated with other organic pollutants. The untreated wastewater effluent that contains pharmaceuticals poses considerable threat to the aquatic ecosystem because of the negative effects of non-target organisms in the water. Recent years have seen a growing concern about the benzodiazepines, as emerging pollutants, and their effects on the aquatic environment. These compounds are nowadays widely detected in sewage wastewater. It is important to increase the emphasis on the characteristics of the benzodiazepines in order to differentiate them from industrial chemical compounds. In this study various solid phase extraction techniques have been employed focusing on isolation of benzodiazepines in wastewater matrices. Employing this methodology has shown improved detection and analysis of diazepam and clonazepam as benzodiazepines.

Preliminary results have shown the diazepam concentrations to range between 1 to 9 ppm in seasonal wastewater samples analysed, with higher concentrations during the winter season. In the case of clonazepam, concentrations ranged between 0.4 to 2 ppm. Seasonal characteristics of the benzodiazepines are discussed along the trends observed for the water quality characteristics of the final treated wastewater.

### 3.14 In Search of the Smoking Gun: Linking Environmental Contamination to its Source

#### 3.14.T-01 A Bird in the Hand: A Sticky Death for Thousands of Seabirds Due to a Spill Of ‘Highly Reactive’ Polyisobutene (PIB).

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Spills of complex mixtures of hydrocarbons other than crude oils can have detrimental effects on wildlife. In January and again in April, 2013, spills of a ‘mystery’ substance in UK coastal waters led to the deaths of thousands of seabirds. We identified the offending substance as a polyisobutene (PIB) by comparison of Fourier Transform infrared (FTIR) spectra with a library spectrum of a ‘conventional’ PIB. However, there were subtle differences between the library spectrum and spectra of the spilled PIB. Examination by high temperature gas chromatography-mass spectroscopy and nuclear magnetic resonance spectroscopy showed that the 2013 pollutant PIB mixture contained oligomers with a high proportion of terminal double bonds, referred to as ‘highly reactive’ PIB (HR-PIB). The identification of the pollutant was finally confirmed by comparison of FTIR and mass spectra with those of an authentic HR-PIB sample. The identification led to a legal re-classification of the potential hazards associated with PIB transport at sea.

#### 3.14.T-02 Can the Source of Oil Pollution be Identified using Diamondoid Molecular Indicators Accumulated in Fish Tissue?

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Following an oil spill, the spilled oil becomes increasingly weathered and identification of its source becomes challenging but can often be achieved by comparing the relative abundances of chemical biomarker compounds known for their resistance to weathering processes, e.g. steranes and hopanes. This is sometimes referred to as oil ‘fingerprinting’. Establishing the source of an oil to which organisms have been exposed is far more challenging, especially in motile species such as fish that also have the ability to metabolize many components of oils. In order to find the ‘smoking gun’, a forensic fingerprint of chemical biomarkers linking the oil components in fish tissues to the source oil is required. An initial step towards achieving this was accomplished using ratios of bicyclic sesquiterpane (bicyclane) isomers to provide characteristic fingerprints of oils conserved in the adipose tissues of fish. However, a limitation of the method was that only six bicyclane ratios were available for comparison and therefore additional forensic ratios are required.

Diamondoids are saturated hydrocarbons with cage-like structures that resemble diamonds. Tricyclic (adamantanes) and pentacyclic (diamantanes) diamondoids have been used as maturity and source rock indicators due to their high thermal stability and resistance to biodegradation. The molecular weight range of such diamondoids (150 – 202 Da for methyladamantanes to methyldiamantanes) are similar to that of the bicyclanes used in the initial study (194 – 222 Da for C<sub>14</sub> – C<sub>16</sub> bicyclanes), suggesting that they may have similar bioavailability and bioaccumulation potential.

In this study, we explore the use of diamondoid indices for their potential for forensic fingerprinting of oils bioaccumulated in fish. In order to enhance resolution of the diamondoids, comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS) was used to analyze the oils and the fish adipose extracts.

Preliminary results correlating indices derived from a crude oil and fish adipose extracts suggest an excellent linear fit close to the ideal 1:1. Additional work is ongoing to test fish exposed to a heavy fuel oil and to explore if the profiles of the indices are sufficiently different for forensic fingerprinting purposes, either in their own right or in conjunction with bicyclane ratios.

### 3.14.T-03 Investigation of Sources of Polychlorinated Biphenyls in Rivers of the Lake Geneva Basin

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In 2018 and 2019, two large-scale surveys of hydrophobic micropollutants were conducted in the Lake Geneva basin.

Polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE) and polycyclic aromatic hydrocarbons (PAH) were studied by deploying passive samplers on over 30 sites in Switzerland and France. Three relevant features (concentration, load and chemical signature of pollutants) were analysed. The results revealed the rivers presenting the highest risk for biota (i.e. highest concentration), as well as those contributing the most to the pollution of the basin (i.e. highest load). Information about the type of PCB source was obtained from the chemical signature.

Three of the rivers presenting a high load or concentration (Chamberonne, Rhône and Nant d'Avril) were chosen for further investigation of their pollution sources. The observed PCB profile (i.e. relative concentration of each sampled congener), combined with knowledge of the geographical and historical context of the river, was used to locate potential pollution sources. Silicone passive samplers were deployed up- and downstream of the river segment in which one or more potential sources were located. A passive sampling-based approach was used as it overcomes the limitations of current approaches targeting hydrophobic micropollutants: insufficient sensitivity, low temporal representativeness and poor data comparability. After identifying the river segment presenting a major increase in concentration and load, as well as a change in chemical profile, the investigation focused on the effluents present in the segment. An example of a relatively straightforward investigation will be given with the Chamberonne river. A river segment presenting a 12-fold increase in PCB concentration and a change in PCB profile was indeed rapidly identified in 2021. Two effluents of a potential source were located in this segment and samplers were deployed closely up- and downstream of the entry points in spring 2022. More complex investigations will be presented through the examples of the Nant d'Avril and the Rhône. In these cases, more sources contributed to the pollution and/or changes in rivers were less marked.

The presented investigative approach, guided by information obtained during large-scale monitoring, can be used to support authorities in the investigation and trial of current pollutant sources, ideally preventing current chemical releases from being continued.

### 3.14.T-04 Time Series Analysis to Elucidate Contamination Sources of Unknown Substances in the Rhine River

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More than 58 million people live along the river Rhine, and many depend on it for drinking water. However, the industrial activity in the Rhine catchment is among the highest in the world, which raises concerns about potential risks from unexpected releases of harmful substances. Aiming to ensure good water quality and to reduce contamination, the monitoring station of the Office for Environment and Energy in Basel is controlling daily intensities of known and unknown substances via liquid chromatography high-resolution mass spectrometry (LC-HRMS/MS), performing target and non-target analysis. This high-throughput, high-frequency monitoring makes it possible to inform downstream drinking water plants in a timely manner if sudden increases in measured signals are observed.

However, to efficiently reduce pollution, it is essential to better comprehend its origin and to trace the main contamination sources. To this end, we performed an in-depth analysis on the monitoring data. After extensive pre-processing, we obtained high-frequency time series containing daily measurements of over 50 000 compounds recorded from 2019 to 2022. In a first step, we identified priority substances of particular relevance for reducing contamination. Secondly, we used state-of-the-art modelling and clustering approaches to identify contaminants originating from similar sources.

The high diversity of time series pattern in the dataset enabled the identification of hundreds of clusters. Based on our models and on information obtained from known substances, we were able to identify multiple clusters with temporal patterns clearly pointing toward one of the possible contamination sources. We detected clusters of substances most probably originating from households, agricultural usage, from contaminated groundwaters or of industrial origin. The chemical structure of several unknown industrial priority substances was identified and those compounds were further monitored with increased vigilance. We observed an overall decreasing trend in contamination, suggesting that measures taken to reduce contaminant load at the watershed level are effective.

In conclusion, we were able to show that, despite the single measurement location, high-frequency monitoring in combination with the use of advanced data science tools allowed obtaining a comprehensive picture of anthropogenic contamination and its sources in the Rhine river, thus providing key information for river surveillance and management.

### 3.14.T-05 Behavioural Fingerprint Implementation for Sourcing Contamination at an Industrial Wastewater Treatment Plant

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Industrial micro pollutants must pass by a wastewater treatment plants (WWTP) before eventual contamination of the environment. WWTPs therefore present a known network point for linking contamination to the source of pollution, which is essential not only from a legal perspective but also for environmental protection. ToxMate biomonitoring, whereby invertebrate locomotor behaviour is tracked in real-time, alerts operators to surges in micro pollutant contamination in effluents. Industrial sites have known upstream production lines that discharge into the dedicated on-site WWTP, which can narrow down the potential sources of contamination. One site, partnered for this study, has been equipped with ToxMate biomonitoring to tackle micro pollution since 2020, and further research was recently undertaken to implement behavioural fingerprinting in the ToxMate biological signal to identify the nature of contamination. The objective of the communication is to present 1/ the observation of micro pollution surges that occur 2/ the strategy used to implement behavioural fingerprinting to help identify types of contamination and thus narrow down potential sources of contamination.

In total, 8 suspected chemicals were targeted by the WWTP and sent to the laboratory for ToxMate testing. In order to implement behavioural fingerprinting at the WWTP effluent for the chemicals, multiple steps were taken: 1/ Organisms were exposed to low concentrations of the chemicals in closed circuit lab conditions 2/ The same exposure was carried out in the effluent bypass 3/ Data screening for the identified fingerprints was analysed for the long-term collected data. Results showed that naturally occurring spikes in micropollutants, that were in turn used for data screening, matched behavioural fingerprints observed during the controlled testing phase. Behavioural fingerprinting, used to assess the probability of a certain contamination nature, was formed using functional data analysis. The behavioural fingerprinting showed potential to provide solution in industrial WWTPs, whereby better understanding of the effluent contamination can point to known sources of contamination.

### 3.14.P In Search of the Smoking Gun: Linking Environmental Contamination to its Source

#### 3.14.P-Th217 Fingerprinting Fish: Using Linear Discriminant Analysis (LDA) of Lipid Bicyclane Profiles to Identify Oil Contamination in Fish

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In the event of an oil spill, identification of source oil(s) for assessment, or for litigation purposes, typically uses diagnostic ratios of chemical biomarkers to produce characteristic oil “fingerprints”. Matching fingerprints from an environmental sample to that of a reference oil is to some extent subjective, and requires expert judgement.

Bicyclic sesquiterpanes (bicyclanes) are a class of petroleum hydrocarbons which have been used to forensically identify fuel oils and light crudes where more traditional biomarkers such as steranes and hopanes are not present. Bicyclanes are sufficiently lipophilic for passive cellular uptake, but are non-toxic, not metabolized by fish, and are resistant to the weathering processes that may alter characteristic fingerprinting ratios beyond use.

In this proof-of-concept study, we conducted a 35-day laboratory trial exposing *Lates calcarifer* (Asian seabass) via diet to either an Australian medium crude oil (Montara) or a heavy fuel oil (HFO). Adipose tissue extracts showed bioaccumulation of bicyclanes in fish lipid tissue with consistent bioaccumulation rates such that diagnostic fingerprinting ratios are preserved. Linear discriminant analysis (LDA) is a form of multivariate analysis similar to principal component analysis (PCA), often applied in facial recognition systems. We trained an LDA model using bicyclane profiles of Montara, HFO and seven other reference crudes, fuel oils and asphaltites. The model was subsequently able to correctly identify the respective exposure oil when challenged with bicyclane profiles from lipid tissue extracts from 18 exposed fish with a posterior probability exceeding 95%.

#### 3.14.P-Th218 Fingerprinting ‘IMO-2020’ Compliant Low Sulphur Fuel Oils using Biomarkers and Heterocyclic Aromatic Compounds

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The International Maritime Organization (IMO), which is responsible for worldwide regulation of the marine sector, mandated that shippers transition to Low-Sulphur Fuels by January 1, 2020. Known as ‘IMO-2020’, its aim is to reduce air pollution, such as sulphur-oxide (SOx) and nitrous-oxide (NOx) compounds and particulates in Emission Control Areas (ECA’s) caused by the burning of marine fossil fuels. IMO-2020 lowered the allowable weight percentage of sulphur from 3.5 wt% down to 0.5 wt%. Fuels compliant with the 0.5 wt% sulphur limit are referred to as Very Low Sulphur Fuel Oils (VLSFOs). In addition to IMO-2020 ECA’s, a number of European Union countries together with the United States and Canada have implemented more stringent Sulphur ECAs (SECAs) requiring ships to utilize marine fuels containing a maximum of 0.1 wt% sulphur, referred to as Ultra Low Sulphur Fuel Oils (ULSFOs). Just eight months after IMO-2020 came into force, the first major spillage of VLSFO occurred when the MV *Wakashio* struck a reef off the southeast coast of Mauritius in August 2020. In order to understand if VLSFOs and ULSFOs can be forensically differentiated, a thorough analysis of their chemical composition is required. In particular, those compounds that are resistant to weathering processes that occur when oil is spilled into the environment. In our study we have examined the chemical characteristics of three VLSFOs and three ULSFOs using traditional GC-MS, comprehensive two-dimensional gas chromatography with high resolution time-of-flight spectrometry (GCxGC-HRT), GCxGC-

FID, and stable isotope analysis by GC-irMS. Comparisons of molecular biomarkers, such as steranes and hopanes, by both GC-MS and GC×GC-FID revealed that the two ULSFOs were near identical, but all other oils could be readily differentiated. Heterocyclic aromatic compounds were analysed by GC×GC-HRT to explore the possibility of developing forensically useful fingerprints using the profiles of their parent and alkylated homologues. Oils could also be differentiated by their  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  of *n*-alkanes and isoprenoids, adding to an overall fingerprint.

The *Wakashio* oil spill may have been the first release of VLSFO into the marine environment but it is unlikely to be the last. This study provides important forensic data that may prove invaluable in the event of future oil spills.

### **3.14.P-Th219 An Annual Resolution 600-year Record of Polycyclic Aromatic Hydrocarbons and Charcoal as Fire Proxies in Laminated Lake Sediments**

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Lakes adjacent to human settlements are storing the relationship between (long-term) environmental changes and human activities (land-use, urban emissions, fire events, etc.). Biogeochemical parameters and pollutant profiles can be associated with pre-industrial production. Such compounds are still eco-toxicologically detectable centuries later. However, publications on lake sediments usually focus on time scales from present to the last ca. 150 years. The multi-disciplinary research project Bad Waldsee (Germany) aims to evaluate the economic-demographic and socio-environmental development of the medieval city Bad Waldsee and its adjacent Stadtsee Lake during the late Medieval period to Modern Times. Here the impact of land-use intensity on the quality of the aquatic system and pollutant emissions archived in the lake sediments were studied systematically, using Polycyclic Aromatic Hydrocarbons (PAHs, 31 compounds) as well as micro- and macro-charcoal particles (as fire proxies) with annual resolution. Macro charcoal results showed 15 screened charcoal peaks, which group into two phases of biomass burning. The first phase in the late Medieval period (ca. 1297-1417 AD; 653-533 cal. yr BP) show high proportions of burned grass and monocot leaves. After more than 200 years probably without severe fire events, in the second phase (ca. 1637-1859 AD; 313 cal. yr BP onwards) wood was the main fire fuel. Indicative PAH concentrations were analyzed for the first burning phase. The obtained results from the geochemical fire indicators support the change in the fuel source. It is reflected by the Dimethyl-Phenanthrene (DMP) isomers ratios and Perylene fluctuations. Moreover, high perylene values at the beginning of the record indicate a biogenic generation process under anoxic sediment conditions and suggests delivery of terrestrial organic material by water in this period. The retene pattern, as a marker of burned soft wood, indicate an increasing share of coniferous biomass along the grass-wood transition in fuel source. The pattern of typical PAH as combustion results ( $\Sigma\text{Fth}$ , Pyr, BaA, Chry, BkF, BeP, BaP, Indeno, BghiP) confirms the low intensity but frequent burning events, showed by the micro-charcoal record. A ca. 10-fold increase of the 6-ring Benzo(ghi)perylene in ca. 1637 AD coincides with the detected high magnitude charcoal peak during the Thirty Years' war, most likely due to amplified macro charcoal input from regional burning events.

### **3.14.P-Th220 Identify a Possible Source Using an Oil Spill Model**

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The Dutch part of the North Sea is one of the most intensively used seas in the world. There are international routes passing along the coast and shipping routes to and from Rotterdam, Antwerp, Zeebrugge, Amsterdam, Eemshaven/Delfzijl. Rijkswaterstaat (the Dutch maritime and marine management organization) is the national authority and is responsible for and activates counter-pollution measures in offshore and inland waters as well as on the shoreline.

In the beginning of 2022 oil tar balls beached over a large area along the coast of the Netherlands and even on beaches of Belgium. Due to weather conditions and technical problems the oil spill wasn't detected by EMSA CleanSeaNet satellite service. The beaches were cleaned and samples were taken from the different beaches for analysis. Oil fingerprinting by GC-FID-MS has identified that these oil tar balls came from the same source. With help of an oil spill model a back track was performed which identified a potential source of the spilled oil.

### **3.14.P-Th221 Low-Cost Passive Air Samplers for High Density Field Deployments in Population-based Studies**

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There is an urgent need to better understand population exposures to airborne chemical emissions, the indoor-outdoor continuum and in areas in close proximity to exposure sources, such as brownfield sites. Significant political motivation exists to develop these sites across Europe to meet housing demand and limit urban sprawl. Concerns of adverse health effects have been expressed by local communities during the remediation and redevelopment, due to potential fugitive emission exposures. The aim of this work was to design multi-functional and novel low-cost passive air samplers (PAS) that can be deployed at-scale in these areas to monitor volatile and semi-volatile organic compounds (VOC/SVOCs). These PAS were milled from PTFE (less than 3 cm in diameter) and contain up to five x 9 mm sorbent discs including (a) a liquid phenylene oxide-coated PALLFLEX® Emfab filter and (b) a polydimethylsiloxane (silicone) substrate. A proof-of-concept trial was performed involving deployment of PAS weekly for a month in the kitchen, bedroom and front garden (roadside) of an urban home. PAS sorbents were extracted and analysed by gas-chromatography-mass spectrometry with >135 identified SVOCs and VOCs tentatively identified using NIST17 libraries. The PAS demonstrated on average a 2-fold accumulation over the month with the most chemical residues found in the bedroom (>forty-five compounds). The chemical fingerprint between the indoor and outdoor environment was evident. The outdoor samplers shared eight common compounds with the indoor samplers, including human and plant metabolites (e.g., octadecane, tetradecane). Within the home, the bedroom and kitchen had nineteen common compounds including personal care products (e.g.

lauraldehyde) and fragrances (e.g. n-hexyl salicylate). These novel PAS show promise for VOC and SVOC monitoring discretely in both public spaces, inside homes and worn as badges. This will complement planned future biomonitoring work that will generate a *weight of evidence* towards an individual's exposome living near or on these brownfield sites.

### **3.14.P-Th222 The Use of Passive Samplers to Monitor Polar Organic Micropollutants in Surface Water and to Produce Knowledge on Contamination Sources**

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Environmental agencies have been addressing the problem of polar organic micropollutants (e.g. pesticides and pharmaceuticals) in surface water for some time now. However, the data collection is seldom systematic over time and space, mainly because of politics and budget restrictions, which makes it difficult to detect relevant patterns. This study proposes to use forensic science theory to design systematic and coordinated monitoring campaigns, and therefore better detect and track problematic situations. Intelligence-led processes aim at acquiring knowledge based on forensic traces to better understand spatial and temporal patterns of water pollution, and to identify chronic pollution sources. Intelligence can be produced from raw data to lead decision and policy makers design adequate actions to significantly reduce pollution problems.

Here, we present a practical low scale monitoring example based on Chemcatcher-like hydrophilic samplers (SDB-RPS extraction disks covered by PES microporous membranes) co-deployed with silicone disks spiked with performance reference compounds. The samplers were calibrated to follow the time-weighted average concentration of 44 hydrophilic contaminants in water. Each device was implemented in the field during 20 consecutive 14-day periods to measure the pollutants' concentrations at 5 different sampling sites. All acquired data (concentrations of pollutants) was added to a structured memory, that also included data from the local environmental agency collected with an autosampler (close to two of the five monitored sites). Sub-problems (trends, patterns) were studied using spatiotemporal visualisations and analyses. The data analysis is also used to guide further the sampling of future monitoring campaigns.

### **3.14.P-Th223 Reconstructing Temporal PFAS Trends from Sediment Cores with Multiple Approaches**

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The use of Per- and polyfluoroalkyl substances (PFAS) in industrial applications predates analytical techniques for their identification in environmental matrices, leaving a gap in historical contamination records. One way to determine past PFAS contamination is through examining sediment cores. In this study, a sediment core was taken from an urban river in Rhode Island, USA. The sediment core was collected in a quiescent depositional area of a dammed mill pond near former mills that were suspected of using products containing PFAS. The core was radiometrically dated using <sup>210</sup>Pb and <sup>137</sup>Cs markers and analyzed for PFAS using multiple techniques including Targeted Analysis (TA), Total Oxidizable Precursor Assay (TOP), Extractable Organic Fluorine (EOF), and Nontarget Analysis (NTA). Results from NTA and radiometric dating were compared to determine co-occurrence of PFAS as a means of source identification.

A constant rate of supply (CRS) model of 0.99 cm/yr was determined from results of radiometric dating. The CRS model was applied to the sediment core sample depths to establish an age-depth model. Initial TA sediment concentrations showed temporal trends of PFAS preserved within the sediment record ranging from <1-55 ng/g sediment (d/w) in 18 unique PFAS compounds. A fluorine mass balance was performed pre- and post TOP assay to determine the presence of PFAS precursors. Results from the mass balance were compared with results from EOF. Certain parts of the core saw as little as 6% of the fluorine accounted for in fluorine mass balance. NTA data revealed the presence of additional PFAS including long chain PFCAs (C15-19), fluorotelomer carboxylic acids (PFCAs), and 6:2/8:2 FT diPAP. The majority of PFAS compounds detected in initial NTA results indicated peak PFAS inputs from 1970-1973.

This research highlights the utility of a multi-analysis approach to characterize temporal PFAS trends in sediments. This study highlights the utility of combining NTA with radiometric data and other proxies to elucidate temporal trends of environmental contaminants. Hierarchical clustering distinguished several notable time periods of PFAS deposition. Many detected PFAS compounds were released into the environment in the mid-1970s. There were also groupings of PFAS released more recently (2000s) and in the mid-1950s. Understanding the depositional history of environmental PFAS is important for understanding PFAS fate and long-term persistence in the environment.

### **3.14.P-Th224 Development of a Groundwater Toolbox to Support Site Conceptual Models**

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The Farm to Fork strategy and Chemical Strategy for Sustainability both identify a need to ensure that the production of food in EU agriculture needs to be conducted in a sustainable fashion to reduce pollution from pesticide use. In order to identify potential emerging contaminants of concern, groundwater monitoring plays an important role in clarifying and identifying potential pollutants to support sustainable use. Whilst being an incredibly powerful tool for driving our understanding of potential water pollutants, it must be recognised that there is a requirement for the development of a toolbox to ensure that groundwater observations are reliable and reflective of environmental processes.

As a starting point, a site visit is always recommended as it must be recognised that damage can, and will, occur for monitoring infrastructure *in situ*, especially when its location brings it into proximity with farming machinery. Therefore, a through site inspection, including an investigation down the monitoring well to look for potential root ingress, cracks or other damage which impacts well integrity. Such events manifest in the analytical data through very high concentrations of the active substance with

no or limited generation of metabolites, especially where these would be expected to form, demonstrating limited contact with the soil and thus potential for biotransformation.

It is also possible to investigate potential flow pathways and sources of water through the analysis of “chemical fingerprinting”. By collecting water from various potential sources at a location (e.g., rainwater, surface water, etc.) it is possible to establish their individual chemical composition, which allows for the relative contribution of each source to a mixed water source to be determined. Further, through the use of aging techniques and flow mapping pathways, it is possible to evaluate potential sources of pollution for the observed observation at a given monitoring well, specifically by establishing water age, travel time and the theoretical zone of recharge.

Overall, groundwater monitoring is a hugely powerful tool for evaluating groundwater health and identifying residues of concern or emerging substances of concern. However, it must be acknowledged that false positives do occur and that a set of techniques for elucidations are required to support data interpretation and support conclusion making.

### 3.14.P-Th225 Impact of Wastewater Treatment Works’ Effluent on Downstream Water Quality

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The presence in wastewater treatment works’ (WwTW) effluents of a range of trace substances is of increasing concern. It is often the default assumption that observed poor river water quality downstream of a particular WwTW discharge arises primarily as a consequence of that specific sewage effluent input. This leads to calls for the implementation of local remedial measures, when in fact the influence of the local discharge is often of limited importance because water quality upstream is already poor. This poster presents data on the influence of local WwTW effluent discharges on water quality. It shows that the sources of contamination in many cases might need to be addressed on a wider river catchment scale rather than on a local basis. Only by drawing this distinction can it be possible to prioritise remedial measures that are more likely to be successful in achieving environmental goals.

**Investigations:** This study uses data from the UK Water Industry Research Chemicals investigation (CIP) programme to examine local impact of effluent discharges. The CIP monitoring programme, gathered over a recent 5-year period, is a resource of water and effluent quality data for over 600 WwTW sites for a total of 50 different determinands.

**Results:** Initial correlation analysis of the CIP database of riverine contaminant concentrations upstream (u/s) and downstream (d/s) of WwTW discharges was carried out for each discharge. Higher correlation tends to indicate low impact i.e. no marked change in concentration from u/s to d/s concentration. Lower correlation indicates an impact by the local effluent such that d/s concentration values diverge from u/s ones. As expected, this divergence is evident for the principal effluent sanitary parameters (COD, BOD, phosphate, ammonia, suspended solids) and also less predictably for a limited number of trace contaminants.

Further analysis will be provided for substances of particular interest of: 1) The change in observed concentrations for the main contaminants of regulatory interest under the Water Framework Directive from u/s to d/s locations, and, 2) The impact that this change in concentration has on compliance with relevant water quality standards downstream of the effluent discharge.

### 3.14.P-Th228 Understand How Distinct Seabird Foraging Strategies Can Influence Mercury Loads Along a Gradient of Exposure

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Mercury (Hg) is a non-essential trace element that can be easily transferred to marine food webs, bioaccumulates along time, and biomagnifies with increasing trophic level. As top predators, seabirds have been extensively used as bioindicators of Hg contamination in the marine environment. According to their foraging strategy, seabirds can be good models to assess the Hg contamination among habitats (e.g., terrestrial, coastal, or oceanic), and they can be relevant to identify local sources of Hg and to biomonitoring the risk of Hg contamination, particularly during the breeding season. Thus, the main goal of the current study was to identify the influence of differential feeding ecology (proxied by  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values) and spatial habitat use (proxied by tracking logger data) in explaining inter-specific and inter-colony variation of blood Hg concentrations along a gradient of exposure. For this, yellow-legged gulls (*Larus michahellis*) were used as bioindicators of the urban/terrestrial and coastal habitats, Audouin’s gulls (*Ichthyophaga audouinii*) were used to assess contamination levels in coastal marine habitats, and Cory’s shearwaters (*Calonectris borealis*) were used as indicators of oceanic habitats. Higher concentrations of Hg were associated to a higher trophic level (higher  $\delta^{15}\text{N}$ ) and to a higher reliance on marine resources (higher  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$ ), strongly supported by the habitat use strategy of yellow-legged gulls in different colonies, and to a lower extent by Audouin’s gulls and Cory’s shearwaters. Breeding Audouin’s and yellow-legged gulls in natural colonies had Hg concentrations above the international defined toxicity thresholds, indicating a potential health risk to these gull populations. The higher reliance on fishery discards by yellow-legged and Audouin’s gulls breeding in the natural colonies, together with the strong dependence on terrestrial resources by urban-dwelling yellow-legged gulls, should explain the Hg patterns found in this study. Although the habitat use strategy helped to understand the variations in Hg levels between yellow-legged gull breeding in different colonies, it failed to show the existence of a oceanic-coastal-terrestrial gradient of exposure to Hg contamination. Finally, we argue for the combined use of stable isotopes and GPS tracking in order to provide a more detailed picture on the characterisation of Hg sources and other contaminants in seabirds.

### 3.14.P-Th229 Using Pelagic and Coastal Seabirds to Compare Chemical Contamination Between the Northeast Atlantic Ocean and the Western Mediterranean Sea

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The continuous growth of anthropogenic activities makes chemical pollution a significant threat to marine environments worldwide, with increasing trends for the upcoming decades. However, the impact of marine pollutants on marine organisms may differ between oceanic basins, associated with differences in specific ecological or geographic features. Our objective was to investigate the differences in the concentrations of metals and metalloids (Arsenic, Bromine, Cadmium, Chromium, Mercury, Nickel, Lead, Strontium, Titanium, Copper, Iron, Selenium and Zinc) between two interconnected but very contrasting basins, the northern Atlantic Ocean and the western Mediterranean Sea. We analysed all these elements in feathers of four seabird species (Cory's shearwater *Calonectris borealis*, Scopoli's shearwater *Calonectris diomedea*, Audouin's gull *Icthyaetus audouinii* and yellow-legged gull *Larus michahellis*) inhabiting these basins during two consecutive breeding seasons (2020 and 2021). Moreover, stable isotope values were also analysed to infer about the trophic level and habitat use of these species, and relate with potential contamination in the two regions. Results showed differences in elements concentrations between the two environments and among species, with habitat use and trophic ecology of seabirds being the main possible cause for such variation. This study reveals the importance of biomonitoring marine environments using top predator species as sentinels to evaluate contamination patterns and dynamics in marine food webs and, ultimately, to maintain the quality of resources and ensure organisms well-being.

### 3.14.P-Th230 Sharing a Success Story: How Access to Reliable Data Is Central to Improved Environmental Performance

**Alexandra Duguay**, Rio Tinto, Canada

As environmental specialists, we are all very familiar with the paramount importance of reliable data. Not only can they support informed decision and adequate management to reduce footprint and environmental damage, but they can help us achieve our goal towards impeccable ESG credentials.

In Saguenay- Lac Saint Jean, Rio Tinto operates an important hub of aluminium assets. These include an alumina refinery, five smelters, six hydropower plants, a high-tech research and development center as well as an operational center. Also, a 300 km rail network connects our international port to the rest of this hub.

With the most stringent environmental protection commitment at heart, we have established a comprehensive surface and underground water monitoring network. It consists of more than 200 monitoring stations equipped with the latest technologies in terms of online telemetry. Every key parameter is monitored including pH, conductivity, organic and inorganic pollutants, as well as heavy metals. This management is done in collaboration with our long-lasting partner: SNC Lavalin.

Previously, and before we integrated with the ESRI suite: Survey 123 and Arc GIS, all environmental monitoring results were received by email via pdf and only after the completion of each campaign. All the results were then re-entered and re-processed manually by Rio Tinto's employees.

Now, using the current flexible cloud to cloud system from the ESRI ecosystem, we are directly connected to the SNC Lavalin database and are receiving our results instantaneously. We gain efficiency, keep ownership of our data as well as accessibility for all users pre-registered to the database. This adds a lot of value and reduces inefficiency because we are no longer spending hours to re-transcribe the data into our own system. We have replaced unreliable paper-based data collection with a trustworthy digital solution that fits the needs of our environmental commitment. This connection will now serve as a foundational element and accelerator for our digital transformation whereby we can envision seamless data integration with all our consultants and data providers, improving efficiency and quality.

With this case study, we will showcase how we have succeeded in creating a positive change taking on the challenge of better sharing environmental data directly from the source and how we were able to implement a sustainable solution to optimize environmental performance in nowadays digital world.

### 3.14.V In Search of the Smoking Gun: Linking Environmental Contamination to its Source

#### 3.14.V-01 Contamination Sources Drive Hg Bioaccumulation in Arctic Marine Predators

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Poor understanding of mercury (Hg) sources and exposure pathways limits the effectiveness of prevention and mitigation of Hg marine pollution. In the Arctic, spatiotemporal variation in monomethyl-Hg (MMHg) production defines Hg bioavailability and trophic biomagnification, dictating Hg exposure in marine predators. Hg stable isotope analysis enhanced our understanding of environmental Hg dynamics. In this study, we aimed at assessing spatiotemporal variation in Hg sources to Arctic marine predators. We measured  $\delta^{202}\text{Hg}$ ,  $\Delta^{199}\text{Hg}$  and  $\Delta^{200}\text{Hg}$  values as well as THg, MMHg and iHg concentrations in muscle ( $n = 483$ ) of hooded seal *Cystophora cristata*, harp seal *Pagophilus groenlandicus* and ringed seal *Pusa hispida* sampled in the Greenland Sea

between 1985 and 2019. We identified Hg sources at a spatial scale, estimating Hg multi-isotopic niches in R (SIBER). We compared THg with  $\delta^{202}\text{Hg}$ ,  $\Delta^{199}\text{Hg}$  and  $\Delta^{200}\text{Hg}$  temporal trends to assess how shifting Hg sources affect Hg bioaccumulation over time. Ringed seals presented the highest THg concentrations ( $1190 \pm 488 \text{ ng.g}^{-1} \text{ dw}$ ), followed by hooded ( $881 \pm 942 \text{ ng.g}^{-1} \text{ dw}$ ) and harp seals ( $407 \pm 289 \text{ ng.g}^{-1} \text{ dw}$ ). The proportion of MMHg did not differ among the three species (74 - 94%). The proportion of iHg was significantly higher in ringed seals ( $14 \pm 4.3\%$ ) than in harp ( $11 \pm 2.7\%$ ) and in hooded seals ( $10 \pm 2.9\%$ ). This was related to ringed seals residing in fjords, where MMHg production and inputs are enhanced by bacterial activity and freshwater run-off. Hg multi-isotopic niches confirmed that ringed seals were exposed to a mixture of terrestrial and local Arctic Hg sources, while harp and hooded seals were more exposed to oceanic and long-range Hg sources. Ringed seal THg varied strongly in time following  $\delta^{202}\text{Hg}$ ,  $\Delta^{199}\text{Hg}$  and  $\Delta^{200}\text{Hg}$  trends, suggesting that Hg bioaccumulation in this species is governed by the complex environmental changes in Arctic coastal marine ecosystems. In hooded seals, THg showed a significant decrease ( $1.5\% \cdot \text{y}^{-1}$ ) over time, following the trends in Atlantic cod and Greenland halibut biomass. This suggests that Hg bioaccumulation in this species is governed by shifts in prey availability. No temporal trends were found for  $\delta^{202}\text{Hg}$ ,  $\Delta^{199}\text{Hg}$  and  $\Delta^{200}\text{Hg}$  values in hooded seals, indicating no shift in Hg sources over time. We conclude that in Arctic seals contamination sources drive Hg bioaccumulation and temporal trends at a species-specific and local scale resolution.

### 3.15.A Innovations in Analytical Methods Used for Monitoring Emerging Contaminants in Marine and Freshwater Aquatic Environments

#### 3.15.A.T-01 A Novel Chemcatcher-Based Method for the Integrative Passive Sampling of Hydrophilic Micropollutants in Surface Water

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Integrative passive sampling is a particularly useful alternative or complementary method to automated active sampling for the monitoring of multiple sites. However, the uptake on the samplers – and thus the sampling rates  $R_s$  – is influenced by environmental factors such as the hydrodynamic conditions. As  $R_s$  are used to calculate the concentration of contaminants in water, it is necessary to have a robust method that accounts for the influence of hydrodynamics. In this study, a novel method relying on the determination of the mass transfer coefficient of the water boundary layer  $k_w$  is proposed using Chemcatcher-like hydrophilic passive samplers.

Chemcatcher-like samplers (SDB-RPS extraction disks with PES membranes) were calibrated at 4 water velocities (5– 40  $\text{cm s}^{-1}$ ) in a channel system for 44 hydrophilic micropollutants. The  $R_s$  were modelised as a function of  $k_w$  to establish relationships between the two parameters. Then, the deployment of a device consisting of a Chemcatcher-like hydrophilic sampler and a silicone disk spiked with performance reference compounds (PRC) was tested in a river during 11 consecutive 14-day periods. The dissipation of the PRC from the silicone disk allowed the measurement of in-situ  $k_w$ , and to determine the most appropriate (site-specific)  $R_s$  from the  $R_s$ – $k_w$  relationships. The obtained time-weighted average (TWA) concentrations were compared with concentrations obtained by automated active sampling (14-day composite samples).

The same compounds were generally detected and quantified using both sampling approaches, though passive sampling achieved lower LOQs because analytes were pre-concentrated in the sorbent. Most measured concentrations (76%) showed no major difference between the two methods. The added value of the PRC-spiked silicone disk was assessed by comparing results with concentrations calculated from fixed  $R_s$  (e.g.,  $R_{s,\text{max}}$ ). The errors on TWA concentrations due to the use of fixed  $R_s$  (instead of site-specific  $R_s$ ) were determined to provide – for each compound – the approximate values of water velocity below which the added value of the method is significant. This showed that the added value was relatively limited in the studied hydrodynamic conditions (24  $\text{cm s}^{-1}$  on average) and that the use of a fixed  $R_s$  is acceptable when working above a given water velocity ( $>20 \text{ cm s}^{-1}$  for 33 compounds). These findings would allow practitioners to assess the best strategy to adopt when monitoring campaigns need to be implemented.

#### 3.15.A.T-02 Water-Soluble Plastics: An Emerging Concern in Freshwater Environments

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In the field of plastic pollution, water-insoluble plastics such as micro and nanoplastics have moved to the forefront of polymer research. Over the last decade, numerous studies have highlighted their detrimental environmental impact, with interest in this field only growing. However, in the wider conversation regarding plastic pollution, water-soluble plastics (WSPs) have been excluded from the conversation.

With a large variety of properties, the applications for WSPs are increasing. Their use in industry as well as household products, often as thickeners, stabilisers and flocculants, demonstrate their prevalence in high-use products and, therefore, their likelihood to end up in freshwater environments, particularly through the route of wastewater treatment.

However, the gap in sensitive analytical techniques to detect WSPs represents one of the greatest challenges to research. Mass spectrometry, such as matrix-assisted laser desorption/ionisation (MALDI), is one of the most explored techniques for polymer research due to the small amount of analyte needed. However, it requires complex sample preparation and is not quantitative. Size-exclusion chromatography (SEC) such as gel-permeation chromatography (GPC), on the other hand, can quantify polymer in the sample but is less sensitive.

As well as difficulties detecting WSPs in the environment, very little is reported on their effects on freshwater organisms. As the major path for WSPs entering the environment is wastewater treatment, a large recipient of this waste is expected to be rivers. Despite this, and due to assumptions made about removal and degradation, there has been little investigation into toxicity and environmental impact to date.

Our research has focussed on developing MALDI-TOF mass spectrometry methods to detect a range of WSPs at a low concentration in samples such as cosmetic products and their degradation in wastewater. As well as this, we have been developing a tandem MALDI-TOF/GPC method to identify, separate and quantify polymers in cosmetic samples and environmental media. Combining these analytical methods with toxicity studies allows sublethal effects to be measured and polymer chemistry and degradation to be measured at the same time, opening up a new avenue in WSP research. This will further our understanding into the scope of these plastics in the environment, as well as their potential threats.

### **3.15.A.T-03 Passive Sampler Design Determines Polar Micropollutant Adsorption and Subsequent Bioassay Responses**

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The combination of integrative passive sampling and bioassays is a promising approach for monitoring the toxicity of polar organic contaminants in aquatic environments. However, the design of integrative passive samplers can affect the accumulation of compounds and therewith the bioassay responses. The present study aimed to determine the effects of sampler housing and sorbent type on the number of chemical features accumulated in polar passive samplers and the subsequent bioassay responses to extracts of these samplers. To this end, four integrative passive sampler configurations, resulting from the combination of polar organic chemical integrative sampler (POCIS) and Speedisk housings with hydrophilic-lipophilic balance and hydrophilic divinylbenzene sorbents, were simultaneously exposed at reference and contaminated surface water locations. The passive sampler extracts were subjected to chemical non-target screening and a battery of five bioassays. Extracts from POCIS contained a higher number of chemical features and caused higher bioassay responses in 91% of cases, while the two sorbents accumulated similar numbers of features and caused equally frequent but different bioassay responses. Hence, the passive sampler design critically affected the number of accumulated polar organic contaminants as well as their toxicity, highlighting the importance of passive sampler design for effect-based water quality assessment.

### **3.15.A.T-04 Occurrence of Wastewater-borne Contaminants in Wild River Fish Tissues by ULPC-HRMS/MS**

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Contamination of surface water sources by wastewater-borne contaminants has recently been evaluated. Aquatic biota, such as wild river fish, are constantly exposed to these compounds and can uptake and accumulate them in their tissues through the skin, gills or diet. Some wastewater-borne contaminants have been previously reported in fish muscle or whole homogenized fish, but few studies have evaluated their distribution in fish tissues. Among the main reasons, muscle analysis is of particular interest in studies investigating the risk assessment of human exposure through the consumption of contaminated fillets. Furthermore, it is easier to obtain a sufficient sample than other tissues that could provide more information. Under this scenario, we analyzed tissues from 4 wild river fish species: liver, kidney, skin, brain, muscle, plasma, heart, and bile to evaluate the distribution of 1556 wastewater-borne contaminants. The extraction method was ultrasound-assisted solvent followed by a clean-up step with Z-sep C18 sorbent. A suspect screening of 1556 analytes was performed. Chromatographic separation and data acquisition were performed via UPLC coupled to a hybrid Q-TOF-MS system and SWATH data acquisition was performed. Thirty-two compounds were detected, of which the majority were PhACs. Of these, 16 were detected at confidence level 2. a (amantadine, bisoprolol, citalopram, clozapine, desmethylocitalopram, fempromimorph, o-desmethylenlafaxine, ondansetron, quetiapine, tiamulin, tramadol, trazodone, Terbutryn, terazosin, triphenyl phosphate, and telmisartan) according to the Schymanski scale, and 16 (clarithromycin, codeine, diltiazem, fluoxetine, metoprolol, sertraline, sotalol, venlafaxine, acetaminophen, acridone, caffeine, carbamazepine, diazepam, loratadine, trimethoprim, and ketoprofen) with confidence level 1 with genuine standards. Of the latter, concentrations were calculated. The presence of sertraline in all tissues was highlighted (C. max 1165 ng g<sup>-1</sup> pancreas). Finally, since most of the confirmed compounds were PhACs, an extraction method was validated to include 83 PhACs in routine analysis. The method was validated at two concentration levels, obtaining recoveries between 40%-120% and RSD <30% for most analytes. In future studies, the validated method will be used to evaluate the bioaccumulation and PhACs distribution in the tissues of 10 common carp, one of the species in which the highest average concentrations were reported.

### **3.15.A.T-05 Application of Compound-Specific Isotope Analysis (Csia) In Identifying Sources and Transformation Mechanisms of the Main Synthetic Musk Compound (Galaxolide)**

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The knowledge of isotopic fingerprints of the environmental organic compounds is of paramount importance in evaluating the fate of organic compounds allowing to trace the source and address transformation processes. Galaxolide (HHCB) is the most frequently used synthetic musk compound, which is attributed to the chemicals of the highest concern. However, despite being

life-threatening, its environmental fate not yet fully known. In this study, we investigated the degradation potential of HHCB and its isotopic fractionation using compound-specific isotope analysis (CSIA) during photodegradation and biodegradation in the aquatic ecosystem. Photodegradation experiments were carried out to investigate direct and indirect photolysis under natural (solar irradiation) and treatment process (UVC irradiation) in synthetic and riverine water including nitrate ( $\text{NO}_3^-$ ), carbonate ( $\text{CO}_3^{2-}$ ), humic substances associated with different experimental conditions, and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in relation to the treatment process. Pure bacterial strain that has been previously isolated from marine canyon sediment was cultivated to test HHCB microbial degradation. Concentrations and carbon isotopic signatures were determined by GC-MS and GC-C-IRMS, respectively. The results have demonstrated that HHCB was quickly photodegraded by direct and indirect photolysis in a range from  $40 \pm 4$  to  $202 \pm 29$  minutes half-life time. Enhanced degradation was observed in the presence of  $\text{H}_2\text{O}_2$  under UVC irradiation due to hydroxyl radicals. Up to 90% of the HHCB depletion driven by a bacterial strain which belongs to genus *Bacillus* sp. was recorded with  $368 \pm 95$  min half-life time. Although a high degradation of HHCB was obtained, no significant isotopic fractionation was determined neither due to photodegradation nor biodegradation. These results reveal that carbon isotopic composition in HHCB is stable against different degradation processes. Isotopic fingerprinting of HHCB can thus be used as novel approach to distinguish the source of HHCB directly from the commercial products and environmental samples.

### 3.15.B Innovations in Analytical Methods Used for Monitoring Emerging Contaminants in Marine and Freshwater Aquatic Environments

#### 3.15.B.T-01 Monitoring Pollution Pathways in River Water by Predictive Path Modelling Using Untargeted GC-MS Measurements

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A comprehensive approach to protect river water quality is needed within the European Water Framework Directive: this requires a paradigm shift from the monitoring of individual target chemicals toward a complete understanding of the aquatic ecosystem. Non-target screening is essential toward this approach, as it provides a complete chemical fingerprint of the aquatic ecosystem, detecting known and yet-unknown chemicals of emerging concern and revealing their suspicious dynamic patterns in river water. Statistic spatiotemporal tools are complementary to track and model such dynamics. A new combination of two measurement paradigms is therefore required: the path of potential pollution should be traced through the river network, while there may be many compounds that make up this chemical composition—both known and unknown. In this work, we combine dedicated data processing of ongoing GC-MS measurements at 9 sites along the Rhine using PARAFAC2 for non-target screening with spatiotemporal modelling of these sites within the river network using path modelling (Process PLS). This new integrated approach allows to track chemicals through the Rhine catchment, to tentatively identify known and as-yet-unknown potential pollutants based on non-target screening and spatiotemporal behaviour, and even to hint toward the source of their contamination. Such an approach utilizes data that has already been collected, allowing water authorities to take effective decisions without adding to the already costly monitoring efforts.

#### 3.15.B.T-02 Unknown PFAS Revealed by the Total Oxidizable Precursor Assay: Towards a Better Understanding of Their Occurrence and Transfers in Marine Biota

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Per- and polyfluoroalkyl substances (PFAS) are thousands of anthropogenic chemicals reported for their ubiquitous presence in the environment. Because of the analytical challenges of the target monitoring of thousands of molecules, scientists have focused on a limited set of PFAS, although an extensive number of overlooked PFAS - including perfluoroalkyl acid precursors - remain unaccounted for. Global understanding of the cycling of PFAS can only be approached through the inclusion of such precursors, for their key role as intermediate compounds of environmental relevance. To better capture this “dark matter”, environmental analytical chemists now have in their toolbox the Total Oxidizable Precursor (TOP) assay, an alternative technique where an additional simple oxidation step turns all the compounds that can generate the stable PFCAs into such easily analyzable final products. With limited marine applications found to date, our goal here is to present how the TOP assay assists our understanding of the cycling of PFAS in marine ecosystems. Two applications will be discussed: their screening in a national marine bivalve biomonitoring programme and the investigation of the trophic transfer of PFAS across coastal foodwebs. First, the occurrence and levels of precursors were assessed in filter-feeding bivalves from the French biomonitoring programme for contaminants, collected annually in 20 sites from 2017 to 2021. In the second application, several species of the European seabass and common sole trophic networks from the Seine estuary, including plankton, polychaetes, bivalves, crustaceans and forage fish, were investigated. PFAS were analysed on one hand via targeted analysis of selected carboxylates, sulfonates and precursors and on the other through a protocol including the oxidative conversion of precursors. Both studies evidenced that PFCA precursors were by far the major contributors to the total PFAS burden in most species, with as little as 10% of the total PFAS concentration being explained by targeted analyses in mussels of the Seine estuary. This presentation will discuss how the post-oxidation levels and profiles assist in gaining insight into the global PFAS contamination of biota. We conclude that conventional PFAS surveys likely miss a large part of the overall PFAS contamination, demonstrating that there is a crucial need to identify the specific compounds responsible for this contamination by means of non-targeted screening approaches.

#### 3.15.B.T-03 Novel Antibody-based Methods for Emerging Contaminant Sensing

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Under the European Union's Water Framework Directive (WFD) (2000/60/EC) all members of the EU are required to protect and improve water quality. The WFD applies to rivers, lakes, groundwater and transitional coastal waters. Traditional detection methodologies involve the use of high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assays (ELISAs).

This project involves the development of a multiplex lab-on-chip (LOC) *in-situ* monitoring system for the detection of harmful algal toxins saxitoxin (paralytic shellfish poisoning toxin) and domoic acid (amnesic shellfish poisoning toxin) and emerging anthropogenic contaminants non-steroidal anti-inflammatory drug (NSAID) diclofenac, the hormone estrone and a member of the pyrethroid insecticide bifenthrin. Competitive immunoassays were generated for each contaminant using 'off-the-shelf' antibodies and in-house generated conjugated contaminants. The immunoassays were then transferred to a lab-on-chip (LOC) detection system for *in-situ* monitoring.

Antibody-based assays were developed to detect each contaminant at or near their required concentrations. Following the development of antibody-based immunosorbent assays, the tests were transferred to the lab-on-chip surface and assay optimisation was performed on-chip. The lab-on-chip surface was composed of poly methyl methacrylate (PMMA) and chip layers were bonded using pressure sensitive adhesive (PSA). Free bifenthrin and diclofenac were tested on plasma treated silanized chips and FITC fluorescence was viewed on a fluorescent microscope, fluorescence intensity was analysed using FIJI software. Assays to detect bifenthrin and diclofenac were performed on the PMMA material. The reduction of fluorescent intensity revealed both free bifenthrin and diclofenac could be detected on the LOCs.

This project is currently on-going and further work is underway to optimise assays for the detection of harmful algal toxins saxitoxin and domoic acid, and the hormone estrone. The work shown herein demonstrates the development of antibody-based immunoassays for the detection of marine emerging contaminants on a novel lab-on-chip platform.

### **3.15.B.T-04 Comprehensive Coverage of Both Known and Unknown PFAS in Ocean, Leachate, and Lake Foam using Non-Targeted Analysis and FluoroMatch 3.0**

Jeremy Koelmel<sup>1</sup>, Emma Rennie<sup>2</sup>, Michael Kummer<sup>3</sup>, Bernard Brooks<sup>3</sup>, Sheng Liu<sup>1</sup>, Carrie McDonough<sup>4</sup>, Emily Parry<sup>2</sup>, David Godri<sup>2</sup>, Jacqueline Bangma<sup>6</sup>, John A Bowden<sup>7</sup>, Nandarani Abril<sup>3</sup>, **Stephan Baumann**<sup>8</sup> and Krystal Pollitt<sup>1</sup>, (1)Yale University, (2)Agilent Technologies, (3)Innovative Omics, (4)Chemistry, Carnegie Mellon University, (5)U.S. Environmental Protection Agency, (6)University of Florida, (7)Academic and Government Applied Marketing, Agilent Technologies

Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) span across thousands to tens of thousands of possible substances. Certain PFAS are persistent, bioaccumulate, and have been linked to various health effects including specific cancers, changes in liver enzymes, reduced immune response, increased cholesterol levels and risk of high blood pressure, slightly lower birth weight, and preeclampsia. These toxicities along with the fate and transport in the environment depend on structure, and only a small portion (< 50%) of PFAS by concentration are generally covered by commonly used analytical approaches.

Therefore, we have developed a non-targeted analysis (NTA) PFAS user-friendly software for comprehensive coverage. FluoroMatch Suite 3.0 performs all NTA data-processing steps: file conversion, peak picking, annotation, scoring, homologous series grouping, and visualization. Recently we have expanded the visualization component for aiding the researcher in discovering unknowns and confirming FluoroMatch annotations as part of our new release. This visualizer shows EICs, annotated MS1 spectra (including isotopic peaks), annotated MS2 spectra, normalized mass defect plots (e.g., CF<sub>2</sub>), tables of annotation evidence and peak areas, and statistical visualizations (PCA, ANOVA, Volcano Plots, Violin Plots, etc.). We have also added new libraries containing MS/MS rules for certain biotransformation products and biotransformation product fragment screening. We applied this software to dried blood spots, aqueous firefighting foams (AFFF), and mice spiked with AFFF. Furthermore, for water bodies, we have found that PFAS concentrate in foams, and have discovered hundreds of PFAS in leachate, lake, and ocean foam samples. Data was acquired on an Agilent 6546 or Agilent 6545, with reverse phase liquid chromatography in negative ion mode. A 5% or less false positive (annotations scored A) and false negative rate was determined for these datasets. PFAS which are uncommonly screened or previously unreported were also discovered including unsaturated pentafluorosulfonic acids and glucuronate PFAS conjugates. PFAS were an order of magnitude or higher in foam compared to representative water samples, with higher chain species being more concentrated in the foam. Foaming water could be a form of remediation of PFAS.

### **3.15.B.T-05 Study of the Influence of Seasonal Conditions on the Occurrence of Pharmaceuticals in Irish Surface Waters**

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Small-molecule pharmaceuticals play an increasingly important role in both human and animal health. The ever-growing use and availability of pharmaceuticals in the last decade have led to the contamination of global surface water ecosystems from ng/L to µg/L concentrations. Additionally, the continuous release, stability and biological activity of these "micro-pollutants" can lead to a "pseudo persistence" in the environment, with ensuing behavioural and health-related effects. However, the occurrence and fate of these pseudo-persistent pharmaceuticals and their residues remain generally unknown. The analysis of 17 pharmaceutical APIs has been accomplished using LC-MS/MS. Eight pharmaceuticals (17 α- ethinylestradiol, 17 β-estradiol, estrone, erythromycin, clarithromycin, azithromycin, amoxicillin, ciprofloxacin) were chosen from the updated surface water "Watch List" (Decision (EU) 2018/840), followed by five pharmaceuticals which are commonly found in European surface water and pharmaceuticals which have a low removal efficiency in conventional activated sludge type wastewater treatment plants (trimethoprim,

sulfamethoxazole, carbamazepine, gemfibrozil and diclofenac). Furthermore, these pharmaceuticals are ubiquitous within the Irish healthcare system (OTC and prescription drugs), are recognised as potentially harmful substances for aquatic organisms and lack Irish data on their presence and concentrations. Surface water grab samples were collected from EPA-recommended sampling locations from September 2020 to March 2022 to provide valuable evidence establishing their presence and identification of their simultaneous occurrence of pharmaceuticals in surface water catchments.

### **3.15.P Innovations in Analytical Methods Used for Monitoring Emerging Contaminants in Marine and Freshwater Aquatic Environments**

#### **3.15.P-We165 Analytical Strategies for Nanoplastics Detection in Complex Biomatrices**

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Micro(nano)plastics are now present throughout the environment, and there is growing concern that their accumulation may, in the future, present serious risks to the environment, including aquatic organisms, and to human health. Although it is a well-recognized problem in the scientific community, the lack of methods to detect the smallest plastic particles (typically < 5 µm) continues to leave huge gaps in knowledge of their distribution and possible harmful effects.

Taking into account the results of previously developed methods on organisms exposed to micro- and nanoplastics, an attempt was made to develop an innovative and cost-effective analytical strategy for the detection of nanoplastic pollution in complex biomass to be applied to routine biomonitoring activities.

In this work, freshwater mussels were used as model organisms, as they are already widely used as bioindicators of plastic contamination. After validating the enzymatic digestion process using metal-doped nanoplastics (recovery greater than 65%), the detection capability of microscopic and spectroscopic techniques was explored. The results obtained indicate that the applied protocol allows the identification and quantification of nanoplastics in the pellet after digestion. In particular, an analytical strategy based on the hydrophobicity of nanoplastics was pursued to adapt routine spectroscopic techniques, such as (µ)Raman, to the detection of plastic particles in complex biomatrices.

#### **3.15.P-We166 Identification of Mid-polar and Polar Aryl Hydrocarbon Receptor Agonists in Gamcheon Harbor of Busan Bay Using Effect-directed Analysis Combined with Full-scan Screening**

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In this study, unmonitored aryl hydrocarbon receptors (AhR) agonists in sediments from Gamcheon Harbor of Busan Bay (S1–S12,  $n=12$ ) were identified using effect-directed analysis (EDA) combined with full-scan screening analysis (FSA). The AhR-mediated potencies were measured in organic extracts, silica gel fractions, and RP-HPLC fractions in sediments using H4IIE-*luc* recombinant cells. Results showed that all sediment extracts showed significant AhR-mediated potencies. Among the silica gel and RP-HPLC fractions, great AhR-mediated potencies were shown in F2.6–F2.8 (mid-polar, log  $K_{OW}$  5–8) and F3.6–F3.7 (polar, log  $K_{OW}$  5–7) in stations S3 and S10. Targeted compounds, including traditional and emerging AhR agonists, could not fully explained the bioassay results (except for the F2.6 of S3 and S10). It is indicated that unknown AhR agonists occurred in the sediments. FSA was performed for F2.7–F2.8 and F3.6–F3.7 of S3 using GC-QTOFMS and LC-QTOFMS, respectively, which showed the great AhR-mediated potencies. Through four-step selection criteria, such as 1) NIST library, 2) library matching score ( $\geq 70$ ), 3) presence of aromatic ring, and 4) number of aromatic rings ( $\geq 4$ ), one (triphenylbenzene) and two (daphnoretin and ishomnetin) tentative AhR agonist candidates were selected in F2.7 and F3.6, respectively. Among the three candidates, daphnoretin and ishomnetin showed significant AhR-mediated potencies, and the relative potency values compared to benzo[*a*]pyrene were  $0.4 \times 10^{-3}$  and  $6.5 \times 10^{-5}$ , respectively. The results of VEGA QSAR, these compounds were confirmed to cause genotoxicity, carcinogenicity, and developmental toxicity. These newly identified AhR agonists have been used for pharmaceuticals. We compared the major AhR agonists in sediments of the highly polluted coastal areas of South Korea. The major AhR agonists in each area differed by region (e.g., chrysene in Ulsan Bay, Lake Sihwa, and Busan Bay; benzo[*b*]fluoranthene in Masan Bay; and benzo[*j*]fluoranthene in Yeongil Bay), which can be attributed to the influence of surrounding activities. The results of this study are significant that provide useful information for the selection and management of the priority substances in the coastal environments.

#### **3.15.P-We167 Integration of Multidisciplinary Techniques to Achieve a Global Diagnosis of a Freshwater Aquatic Ecosystem**

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Freshwater ecosystems are at particular risk because they are continuously exposed to multiple-stressors effects. In addition to pollution and conflicts in the water use, climate change is emerging as a new and increasingly worrisome challenge. At Southern Spain, the Guadalquivir River is the main hydrographic basin of Andalusian region that supports the impact of numerous socioeconomic activities (e.g.: urban centers, and agricultural/industrial/mining activities). With a transversal team made up of researchers from different areas, we have carried out a global diagnosis of the river by using different multidisciplinary techniques

to evaluate biochemical, cellular, physiological and behavioral changes along its course. Significant changes have been found in the concentration of total soluble organic carbon, suspended solids, and in some metals (e.g.: iron), as well as high turbidity in the final stretch of the river. Agricultural activities carried out around the river are responsible for the presence of nitrates and ammonia in certain sections of the river. From the integration of physicochemical and microbiological analysis, a water quality index was evaluated. This index, which was the highest at the source, decreased along the course of the river. Poor water quality values were related to anthropogenic activities carried out in tributaries of the Guadalquivir River (high levels of Fe and Cu and proliferation of coliform bacteria, such as *E. coli*, from the Guadaira and Genil rivers, respectively) or in the final stretch of the river (agricultural activities). Since microorganisms are in almost every environment in the biosphere playing unique essential roles, we have also assessed the utility of water metaomics for environmental diagnosis. The taxonomic identification through genomic analysis of 16S rRNA made it possible to assess changes in the global structure of the microbiome in terms of bacterial distribution, abundance, and diversity along the riverbed, alterations that were associated to variations in physicochemical variables and to environmental stress sources. This multidisciplinary study can be used as a model to prepare risk maps, with the identification of "hot-spots" on which to act, to improve the management of fundamental water resources.

### 3.15.P-We168 Cytotoxicity of Anti-androgenic Endocrine Disrupting Compounds Occurring in the Brackish Baltic Waters

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The anthropogenic pressure causes a constantly increasing problem of toxic compounds influx to the marine waters. These chemicals include endocrine disruptors (EDs), chemicals affecting multiple endocrine pathways and disturbing the physiological reproductive functions of various species, including molluscs. They include a heterogeneous group of chemicals and are divided in two main categories, the estrogenic (xenoestrogens) and anti-androgenic EDs. The exposure to these chemicals has been associated with reproduction disorders impairing fertility and fecundity, intersex and imposex formation, developmental disorders, but also affecting various metabolic pathways etc.

The aim of our study was to assess the seasonal changes in the presence and concentration of chemicals acting as potential EDs occurring in the brackish water and to analyze the possible impact of these compounds using various biological models, including GMM organisms, cancer cell line and *Mytilus trossulus*. The water and bivalves samples were collected seasonally during one-year period in the vicinity of the sewage treatment plant Dębogórze (the Gulf of Gdańsk). The assessment of androgenotoxicity and estrogenotoxicity of sub-bottom water was conducted using the system of genetically modified yeast XenoScreen YES/YAS and the cancer cell line BT-474. Additionally, the transcriptomic analyses were performed based on *M. trossulus* samples to test the effect of EDs using the RNA-seq method.

The obtained results highlighted the presence of EDs compounds characterized by a high anti-androgenic activities. The highest concentrations of the anti-androgen equivalents were noted in the samples collected in the autumn 2021 (36,47 µM aAEQ) and winter 2022 (23,57 µM aAEQ). No substances with androgenic activity were detected. Studies conducted on the cell line BT-474 showed that administration of the water samples induces cytotoxic effects, decreasing cell viability. At the same time the water samples demonstrated no estrogenic or anti-estrogenic activity. Further research concerning the analysis of the transcriptome changes of *M. trossulus* will contribute to the assessment of the potential impact of EDs on the sea bivalves.

### 3.15.P-We169 Industry Related Wastewater of Dialysis Membrane Production Containing N,N-Dimethylacetamid and N,N-Dimethylamin: Screening Combined Toxicity Using *Daphnia magna* and *Raphidocelis subcapitata*

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Dialysis is one of the most important forms of kidney replacement therapy. Wastewater generated during the production of dialysis membranes contains the solvent N,N-dimethylacetamide (DMAc) and its primary degradation product N,N-Dimethylamin (DMA) both known as critical substances for aquatic organism. The project Med-zeroSolvent [1] aims to 1) prevent transfer of these substances via process water to wastewater treatment plants and then the aquatic environment, and 2) the development of an adequate on-site treatment for a wastewater-free production process. Effect-based methods (EBM), such as ecotoxicological bioassays [2], can be applied to evaluate a potential toxicity of DMAc and DMA.

To analyze the ecotoxicity of solvent containing process water, an acute toxicity test using *Daphnia magna* (immobilization) and the combined algae test using *Raphidocelis subcapitata* (inhibition of photosystem II (PSII) and growth) were applied [3,4,5,6]. DMAc and DMA were tested as single compounds, in mixture after concentration addition was calculated and, finally, process water was evaluated.

Assessing individual compounds resulted in an EC50<sub>DaphniaDMAc</sub> of 575 mg/L, EC50<sub>DaphniaDMA</sub> of 34 mg/L and for the mixture in an EC50<sub>DaphniaMix</sub> of 288 mg/L DMAc and 17 mg/L DMA. Therefore, mixture testing in daphnids showed an additive toxicity compared to the single compounds. Testing process water resulted in EC50<sub>DaphniaPW</sub> between a dilution of 1.56% and 3.13%. Preliminary results of the combined algae assay showed low inhibition of growth and PS-II for DMAc and process water. In contrast, high inhibition of PSII and growth was observed for DMA and the mixture. To check the actual concentrations during the experiments DMAc and DMA were analyzed by HPLC or IC methods in exposure media incl. process water.

Generally, it is useful to assess mixtures with EBM [1,7,8]. In this case we detected an additive toxicity when testing the mixture of DMAc and DMA. Contribution of further environmentally critical substances to the toxicity of process water should clarify in future since e.g., bisphenols, polysulfone and N-methyl-2-pyrrolidinone [9] could be found in membrane process water. In Med-zeroSolvent, scalable treatment modules will be developed for process water. Nevertheless, these modules may help to reduce freshwater demand and emissions from comparable industrial processes and wastewaters.

### 3.15.P-We172 Multi-Residue Determination of Pharmaceuticals in *Daphnia magna* and *Danio rerio* Using Solid Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry

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The influx of pharmaceutical mixtures into the aquatic environment poses a significant risk to the aquatic biota. Better understanding of the potential for pharmaceutical compounds to bioconcentrate in biota and determination of the toxicokinetics requires development of reliable analytical methods that can quantitatively measure a wide range of pharmaceuticals with varying physico-chemical properties at trace-levels. We developed, optimized, and validated an analytical method using liquid-chromatography-tandem mass spectrometry (LC-MS/MS) for multi-residue determination and quantification for over 100 pharmaceuticals covering a wide range of therapeutic classes such as antidepressants, NSAIDs, calcium channel blockers, lipid regulators, etc. The method was developed to determine pharmaceutical concentrations in both *Daphnia magna* and *Danio rerio* to enable cross-species comparison of toxicokinetics. The method used solid phase extraction (SPE) for pre-treatment and clean-up followed by LC-MS/MS analysis. Sample clean-up was investigated using different approaches that included freezing-lipid precipitation and tandem SPE with varying sorbents. Additionally, a high-throughput clean-up method was studied using a 96-well micro-elution plate. Overall, the method showed good performance with method detection limits at single figure ng g<sup>-1</sup> range. Quantitatively the method showed acceptable linearity ( $R^2 \geq 0.98$ ) and method repeatability (<15% RSD) across the ng to µg g<sup>-1</sup> range. The method was successfully applied to determine the internal concentration of analytes in *Daphnia magna* exposed to selected pharmaceuticals.

### 3.15.P-We173 Historic Landfills as a Source of Emerging Contaminants to Aquatic Systems

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Many chemicals used by society ultimately end up in landfill sites. It is being increasingly recognised that legacy landfill sites or sites with insufficient management are an important source of contaminants into aquatic systems. However, work to date on the chemical characteristics of landfill leachate has focused on only a handful of chemicals. This study was therefore performed to characterise the chemical signature of landfill leachate from cells of different ages. Monthly leachate samples were obtained from cells of different ages from a large landfill site between March 2020 to February 2021. Leachate samples were analysed using a High Resolution Mass Spectrometry (HRMS) Orbitrap. The resulting data files were searched for 5871 compounds, including current use and banned pesticides, pharmaceuticals, biocides and home-use products, that would be expected to be disposed to landfills. So far, data have been obtained for two sampling timepoints (September and January). A total of 128 chemicals has been identified with the highest number (83) of compounds being detected in a leachate from a cell that was closed in 2016. Only six chemicals were detected in leachate from the oldest cells (closed in 1988) namely DEET (an insect repellent), Selenium Sulfide (an antidandruff treatment), DEHP (a plasticizer), profenofos (an insecticide), N,N'-diphenylguanidine (a rubber vulcanizing agent) and EDTA (a chelating agent used in personal care products). The data analysis is ongoing and will be used to construct a chemical history of the landfill.

### 3.15.P-We174 Targeting Metal Impurities for the Detection and Quantification of Carbon Black Particles in Water via spICP-MS

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Carbon black (CB) is a nanomaterial with numerous industrial applications and increasing use, prompting concern for potential environmental release and accumulation. In order to improve detection capabilities for this and other graphitic materials, particularly in water systems, this study investigated the metal impurities contained within these materials as potential analytical tracers. The analysis of eighteen different metal impurities was conducted on six commercial varieties of CB materials. Of the metals identified in these characteristic metal fingerprints, La impurity was chosen as a tracer for single particle inductively coupled mass spectrometry (spICP-MS) based on the apparent concentration, low detection limits, and lack of polyatomic interferences. Following optimization efforts, a limit of detection of 63.9 µg/L and quantification limit of 122.2 µg/L of CB exemplified the sensitivity of this novel detection/quantification technique. When compared to UV-visible absorption spectroscopy, and gravimetric analysis, this spICP-MS approach exhibited similar quantification sensitivity in the mg/L concentration range, with the ability to detect CB particles an entire order of magnitude lower. The particle sizing capability of the spICP-MS technique allowed for consistent sizing of the most frequent La particle diameter contained in CB, which could act as a characteristic signal for detection of these particles in environmental waters. The sensitivity of this detection method was unaffected when dissolved lanthanum was added to sample matrices at concentrations typical of environmental waters. In comparison to existing technologies, this high throughput method of detection has achieved competitive sensitivity, a wide dynamic working range for quantification, and high selectivity for managing complex matrices.

### **3.15.P-We175 Method Validation for 260 Pesticides Quantitation at ng/L Level in Water samples with LC – ESI Laminar Flow MS/MS and Direct Injection**

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European Union water policies are continuously being reevaluated to determine safe levels of many contaminants in water sources, resulting in the lowering of detection limits by various governments to meet more stringent regulatory requirements. The aim of this study was to develop and validate a LCMSMS method with direct injection in order to analyze a large polarity range of pesticides in water samples. Results needed to meet the protocol (EU reference ISO/TS 13530:2009), based on the French regulations NF-TO-90210. They should also include statistical tests on calibration, robustness and inaccuracy studies.

### **3.15.P-We176 Identification of Contamination Sources in Undergroundwater at Hanlim, Jeju Island by Target/Suspect/Non-target Analysis with LC/Orbitrap-MS**

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Groundwater and subsurface environment pollution from anthropogenic sources has been a serious problem owing to the long-term recovery period and not judging defaulters immediately. To identify the contamination sources, Environmental forensics technology has recently received a lot of attention for identifying contamination sources. In this aspect, target, suspect, and non-target analysis with high-resolution mass spectrometry (HRMS) is regarded as a new tool to decipher the diverse and specific chemical molecular formulas of compounds occurring at trace levels in matrices without reference standards. In addition, related previous research has suggested the use of chemical compounds as indicators or tracers to represent specific major contaminant sources, such as pesticides, livestock wastewater, and industrial activities. This study aimed to screen unrevealed organic contaminants and identify indicator chemicals for agriculture and livestock contamination sources by suspected and non-target screening using LC/Orbitrap-MS. Hanlim in Jeju, which had a pig manure accident history in 2017, was selected as our sampling site to evaluate our technology scheme. In brief, 35 compounds were detected through targeted and suspected screening at Hanlim, and some antibiotics were only detected in major manure-contaminated sites. We are also suggesting certain new indicators for livestock origin by statistical analysis. Detailed information will be provided during the presentation at the conference.

### **3.15.P-We177 Pilot Large Scale Testing of UV LED Technologies for Reuse of Wastewaters: Anti-microbial Efficiency and Effects on Micropollutant Concentrations**

**Lucie Blahova<sup>1</sup>, Michal Bittner<sup>1</sup>, Kumiko Oguma<sup>2</sup>, Jutta Eggers<sup>3</sup>, Tim Schwarzenberger<sup>3</sup>, Jaroslav Lev<sup>4</sup>, Lucie Baborska<sup>4</sup> and Ludek Blaha<sup>5</sup>,** (1)RECETOX, Masaryk University, Czech Republic, (2)University of Tokyo, Japan, (3)TZW DVGW (German Water Centre), Germany, (4)ASIO TECH s.r.o., Czech Republic, (5)RECETOX, RECETOX, Masaryk University, Czech Republic

In the present study, we report results of one of the first large scale pilot study that tested innovative LED reactors emitting UV (UV LED) for disinfection of pre-treated wastewater effluents. Two different UV LED systems were installed in parallel with conventional low pressure Hg-based UV lamp (repeated 7 day-long pilots) at the municipal wastewater treatment plant (WWTP). The investigation showed that UV LED was efficient in reducing numbers of microorganisms in WWTP effluent waters that might be recycled and used for various technological or irrigation purposes. At the same time UV irradiation was observed to affect concentrations of some micropollutants in the final effluents. Pharmaceuticals and other xenobiotics enter the environment through excretion either in their original parental form or as metabolites formed during detoxification in vivo. Most importantly, glucuronide or sulfate conjugates with variable chemical properties may reach receiving waters at concentrations that are comparable or even higher than those of parent compounds. Deconjugation of the metabolites in the environment including WWTPs may release original biologically active pharmaceuticals. This phenomenon was confirmed by LC-MS/MS in the present study, where UV irradiation triggered deconjugation of metabolites followed by unexpected increases in concentrations of parent pharmaceuticals. These observations indicate potential risks for water quality, which needs further investigation.

### **3.15.P-We178 Application of Passive Sampling to Monitor Melamine and Its Derivatives in Water Environment**

**Yoonah Jeong,** Korea Institute of Civil Engineering and Building Technology (KICT), Korea Melamine is one of the emerging contaminants with persistent, mobile, and toxic (PMT) properties. Due to the release of large quantities for various uses, the widespread occurrence of melamine in the environmental matrix has been reported. Once discharged, melamine can be transformed into cyanuric acid, ammeline, and ammelide. In this study, passive sampling was applied to identify the efficient monitoring method to monitor melamine and its three derivatives in the water environment. Based on existing passive samplers, polar organic chemical integrative sampler, and chemcatcher, various sorbents and membranes were applied for sampler materials. As a receiving phase, SDB-RPS disk, SDB-XC, Oasis HLB, and Oasis MCX were employed. In addition, PTFE and PES membranes were prepared. Sampling rates and sampler-water partition coefficients for four target compounds were determined for various passive sampling combinations. The results of this study contribute in situ monitoring of melamine and its derivatives in surface water.

### **3.15.P-We179 Parameter-Free Extracted Ion Chromatography Builder for Non-Target Screening**

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The non-target screening (NTS) is a promising method for aquatic system monitoring and risk mitigation. NTS is mainly a

qualifying method for liquid chromatography coupled with high-resolution mass spectrometry. It does not require reference standards by design and is used for samples with unknown substance compositions like waste waters or rivers. Unfortunately, there is a lack of standardized algorithms for evaluating NTS data, which then provide hardly comparable results. Besides, many NTS algorithms require user-input parameters. Therefore, we are developing a new generation of NTS algorithms that (1) do not require any user-input parameters, (2) provide uncertainties for all intermediate results, and (3) consider uncertainties from former steps for calculations. We have already published a centroiding method for HRMS data that follows our abovementioned concept. We will now present a modern method for extracting ion chromatograms (XIC). Thereby, the new algorithm will work user-independent and considers and provides the process uncertainties.

The algorithm's main principle is considering each data point's  $m/z$  distance to its right neighbour when the dataset has been sorted. If two  $m/z$  belong to the same XIC, their distance will follow a characteristic distribution (Null Hypothesis  $H_0$ ). However, the Alternative Hypothesis ( $H_1$ ) will be accepted if a critical value is exceeded.

The algorithm was applied to various data sets and compared to other XIC building concepts. We calculated the silhouette score for each XIC that describes its data quality. Moreover, we used automatic peak detection to distinguish between XICs containing chromatographic peaks (True Positives TP) and only noise (False Positives FP). We obtain that our new method reaches the highest silhouette scores, i.e., the classification quality was best. Using peak detection, it can be deduced that all methods detect a significant number of FP XICs. However, our approach provides more TPs as well.

The new algorithm presented in this work is a suitable parameter-free alternative to existing XIC builders for NTS. The method uses information from the data and an iterative process to find the best XIC composition for any NTS data set, which fits the urgent demand for harmonized data evaluation. Moreover, the silhouette score is suitable for describing XICs' qualities to improve removing FP from NTS results.

### **3.15.P-We180 Influence of the Sample Pretreatment Method on the Suspect Screening Output of Pollutants Identified in Treated Wastewaters from an Urban/Industrial WWTP**

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In the current global/climate change context, the circular economy as a sustainable model has been applied in the water sector as a solution to mitigate the water scarcity, mainly, in arid and semi-arid areas such as the Mediterranean. However, among other reasons, industrial pollution appears as an obstacle due to the contribution of organic pollutants to the water networks as a consequence of their incomplete removal in wastewater treatment plants (WWTPs). This is the case of a wide variety of pollutants, such as industrial chemicals or pharmaceuticals, which could represent a risk for the environmental and human health. For the identification of these pollutants, the application of suspect screening (SS) methodologies is emerging as a practical, convenient approach. However, the kind of sample treatment applied before the SS analysis can have a large impact on the outcome, leaving out in some cases relevant pollutants. In order to investigate this issue, in the present study, three different pretreatment methods were applied to a series of treated wastewater samples collected daily during one week from an urban/industrial WWTP located in an industrial area: (i) direct injection (100  $\mu$ L), (ii) lyophilization of 250 mL of sample, reconstitution in a final volume of 1 mL of methanol:water 25:75 and injection of 5  $\mu$ L of the solution, and (iii) on-line solid phase extraction (SPE) of 1 mL of water using a polymeric cartridge. The subsequent analysis of the samples was performed in all cases by LC-HRMS using a hybrid Q-ToF analyzer and in positive and negative ionization. Then the SS workflow for identification of the compounds present in the samples considered different parameters such as exact mass, isotopic pattern and fragmentation, as well as the contrast of the data obtained with MS libraries. From the different sample pretreatment methods tested, the lyophilization allowed the identification of the largest number of compounds, probably because in the online SPE procedure some compounds are lost, and in the direct injection method the comparatively lower sensitivity achieved together with the existence of relevant matrix effects do not allow the detection of compounds present at low concentrations or exhibiting low ionization efficiencies. The results of this study also showed the predominant presence of pharmaceuticals and industrial chemicals followed by some personal care products and other categories of substances, such as caffeine or pesticides.

### **3.15.P-We181 Environmental and Multisource Monitoring – Strategies and Approaches in PARC**

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The European Partnership for the Assessment of Risks from Chemicals (PARC) was launched in May 2022 and aims to advance current risk assessment practices, for a better protection of the environment and human health. The monitoring of chemicals in the environment is an important component of risk assessment, also including matrices directly related to exposure, e.g. food and dust. An appropriate design of monitoring studies is a challenging task, which should also consider the high number of potentially harmful chemicals. The aim of the task “Environmental and multisource monitoring” in PARC is to develop an overall framework for a science-based selection of chemicals for environmental monitoring and to design monitoring schemes closely linked to activities to establish an early warning system. This overall framework includes a prioritization mechanism, the review of existing knowledge, the design and implementation of monitoring studies, data analysis and a feedback step. It is based on the two main prerequisites of responding to regulatory needs and of building on existing knowledge and infrastructure. Depending on the questions to be answered, it will include target methods and non-target/suspect screening approaches as well as effect-based

methods. An ongoing pilot project addresses the regulatory relevant compounds classes of endocrine disrupting chemicals (ECD) and per- and polyfluoralkyl substances.

### 3.15.P-We182 Sorption Kinetics of Psychoactive Compounds on Active-Passive Sampling (APS) Sorbent Gels

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The novel environmental pollutant monitoring approach, active-passive sampling (APS), is being optimized for use in wastewater-based epidemiology (WBE). APS provides *in situ* pre-concentration of pollutants in water independent of the hydrodynamic conditions. The APS device incorporates a pump to control the laminar flow of the sampled medium into a diffusion cell which houses sorbent gels that selectively sorb pollutants. In this study, sorption kinetics on sorbent gels of commonly detected antidepressants, opioids, and illicit drugs in WBE was investigated by laboratory exposure experiments in tap water. Agarose- and polymer-based HLB (hydrophilic-lipophilic balanced) and MCX (mixed-mode cation exchanger) sorbent gels were evaluated by exposure to spiked tap water at different exposure times. Results showed that O-desmethylvenlafaxine, morphine, tramadol, and benzoylecgonine sorbed preferentially on HLB while N-desmethylnaloxonium and amphetamine sorbed to a greater extent on MCX; benzoylecgonine was sorbed the least by both sorbents. Sorbent-water partitioning coefficients ( $K_{SW}$ ) were significantly higher on polymer-based sorbent gels compared to the agarose-based counterparts. Accumulation rate constants ( $k_s$ ) varied significantly between MCX gels but not between HLB gels. Exposure at different water conditions (pH and salinity) was undertaken to explore the interplay between the hydrophilic and electrostatic binding properties of the sorbent gels and the physicochemical properties of the target compounds. For HLB, sorption was greatest at pH 8-9 for most compounds while sorption on MCX increased as the pH was decreased. At increasing ionic strength of water (0-3 g/L NaCl), more abrupt decrease in sorption was observed for MCX as compared to HLB. Findings demonstrate that sorption kinetics of psychoactive compounds on sorbent gels depend on chemical interactions in the sampling medium. The complementary use of HLB and MCX sorbent gels is proposed for a wider coverage of different psychoactive drugs in WBE.

### 3.15.P-We183 Screening Of Organic Pollutants In Clam And Fish Samples By Matrix Solid-phase Dispersion Combined To Liquid And Gas Chromatography With High Resolution Mass Spectrometry

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The presence of organic micropollutants (OMPs), such as persistent organic pollutants (POPs) and contaminants of emerging concern (CECs) has been documented in several aquatic environments. The bioaccumulation of these compounds has become a matter of concern for its possible transfer to the food chain and impact on several wildlife species of the marine environment and human consumers of seafood. Thus, the aim of this work was to apply a wide screening strategy in order to detect different types of OMPs in fish and clam samples from the Portuguese coast.

Liver and muscle of *Trachurus trachurus*, *Solea solea*, *Microchirus azevia*, *Microchirus variegatus*, *Trisopterus luscus* and the whole body of *Spisula solida* were collected from different areas of the Portuguese coast within the project CEIC (Contaminants in commercial species from the Portuguese coast). Sample treatment was performed by three matrix solid-phase dispersion (MSPD) methods and then the extracts were analyzed by both liquid and gas chromatography coupled to high-resolution mass spectrometry (LC-HRMS and GC-HRMS).

In this way, a total of 120 OMPs were identified by using two high-resolution and one low-resolution (NIST) spectral libraries. From the substances detected, 58% of them derived from industrial activity, 22% were pharmaceuticals and drugs, 12% cosmetic products and 8% pesticides. Moreover, the number of OMPs identified using the three different MSPD methods was compared. The method that allowed the detection of the highest number of OMPs was the one incorporating silica impregnated with sulfuric acid, likely because of its capacity to remove lipids and other interfering substances.

Among the surveyed samples, clams and fish muscle showed a similar contamination profile regardless of the collection area and the number of OMPs detected compared to fish liver, which exhibited a different profile.

### 3.15.P-We184 Complex Investigation of the Potential Environmental Risk of UV-filtering Compounds in Cosmetics for Lake Balaton

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Our research group investigates the presence of different chemicals in surface waters, performs risk assessment, and investigates the effects on aquatic species. Nowadays, the presence of synthetic UV-filtering compounds in the global ecosystem is a hot topic in ecotoxicology. It has been shown, mostly by recently published studies, that these chemicals can cause acute and chronic toxicity to aquatic invertebrate and fish species.

Lake Balaton (Hungary) is the largest shallow lake of Central Europe and an important summer holiday destination visited by millions of tourist every year. Hence, the preservation of its good water quality and biodiversity is of strategic importance from an ecological and economic point of view. Based on our calculations, about 2.5 tons of UV-filtering substances can dissolve in Lake

Balaton on an average summer day. Importantly, surveys aiming at the investigation of the presence of these compounds in Hungary have not yet been performed. Hence, the aims of my research are to determine what type of UV-filtering compounds are present in Lake Balaton, to investigate their spatio-temporal variations, and to investigate their potential effects on the widely used ecotoxicological model species, the water flea (*Daphnia magna*).

During my experiments, I focus on five UV-filtering compounds, avobenzone, octinoxate, octocrylene, octisalate, and homosalate. After the appropriate sample preparation (e.g., lyophilisation, microwave-assisted, and solid phase extraction), the compounds are analyzed with UHPLC-MS in water, sediment, biofilm, and biota samples. Besides, we performed photostability and, for the first time, freeze-thaw stability experiments under different temperature and pH conditions in both aqueous and non-aqueous solutions with UV-Vis spectrophotometry to ensure a more realistic environmental measurement. Moreover, in preliminary experiments, we exposed *D. magna* specimens to avobenzone, octocrylene, octinoxate at an environmentally relevant concentration (200 ng/L) for 21 days. During the treatments, changes in the heart rate, body size, and reproduction (time of the first egg production, egg number in the first production, and maximum egg number per individual) were investigated. All of the compounds significantly increased all of the behavioral parameters investigated. Our results show that the release of sunscreen agents into aquatic ecosystems poses an environmental risk.

### **3.15.P-We185 Analytical Monitoring of Organic Contaminants Treated by an Industrial Waste Water Treatment Plant for a Deeper Understanding of Removal Rates**

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Wastewater treatment is a key step for minimizing the release of organic molecules into the environment. Through a multistep process, e.g. physico-chemical and biological processes, molecules are removed from waste water effluents and degraded through bioactivity of inoculum. Although routine analyses are frequently performed to monitor WWTP efficiency (e.g. TOC, BOD, pH...), specific analyses at a substance specific level are needed for in-depth understanding of removal processes and to further minimize the release of potential micro-pollutants into surface water.

As a proactive initiative, an inventory of organic molecules in waste water, being treated by an industrial WWTP has been established, with an emphasis on potential micro-pollutants. The industrial WWTP studied represents an on-site treatment plant at a fragrance manufacturing / formulation site. A list of molecules of interest was selected for method development, based on production and consumption rates, their persistency (low biodegradation) and hydrophilic behaviour (low log Kow). Appropriate sample preparation (e.g. LLE or SPE) and analytical technique (e.g. LC-HRMS or GC-MS) were optimized in order to cover a concentration range from 0.01 to 10 ppm.

For the initial study, incoming and outflow effluents of the biological step were sampled on a weekly basis for 14 weeks and analysed by headspace GC-MS for volatiles and SPE-GC-MS for semi-volatiles. This initial assessment highlights the most recurring organic molecules in the incoming & outlet flows and provides qualitative removal rates.

In a second study, quantitative monitoring of selected molecules was carried out through a daily sampling point approach. Quantitative data can be extracted using a targeted analysis approach such as determination of removal and release rates. With daily data collected on a substance specific basis, root cause investigations can be implemented for identifying which specific industrial process(es) mostly contribute to the level of organic compounds found at WWTP.

The data generated by the present study allows more detailed understanding of an industrial WWTP at a substance specific level. It provides novel data which enables the optimization of industrial processes and the development of additional treatment regimes for the more ecotoxic molecules. Finally, it provides a real world case study for potentially refining environmental risk assessment at a fragrance manufacturing and/or formulation site.

### **3.15.P-We187 Development of a Multiresidue Method for the Determination of Pharmaceutical Products in Seawater**

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The increase of population and industrial development have produced an increase on the presence of potentially toxic compounds. These compounds are known as contaminants of emerging concern (CEC), among them the pharmaceutical compounds and their metabolites are highly spread. The main sources of their presence in the environment are, firstly wastes from laboratories, hospitals and agriculture, and secondly their excretion after their consumption, which appears in wastewaters. Some of them are not removed in wastewater treatment plants and consequently they could contaminate seawaters. The aim of this work is to develop a rapid and sensitive multi-residue method using liquid chromatography coupled to mass spectrometry of triple quadrupole LC-MS/MS (QqQ) with SPE on-line extraction for the determination of pharmaceutical compounds in seawaters. 56 pharmaceutical compounds including antibiotics, analgesics, cardiovascular drugs and psychiatric drugs among others were determined. The proposed method injects only 100 µl of water sample as alternative to the pre-concentration phase allowing to quantify the majority of analytes in the range of <10 ng/L in a short period of time, due to these characteristics it is appropriate for the monitorization of environmental samples.

### **3.15.P-We188 An Automated Toxicity Based Prioritization Framework for Fast Chemical Characterization in Non-Targeted Analysis and Its Validation in Sludge Water**

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The identification of environmental pollutants with harmful effects is usually performed by non-targeted analysis (NTA) using liquid chromatography coupled with high resolution mass spectrometry. However, the prioritization of possible candidates is challenging due to the large number of candidates from MS acquisitions. Therefore, we have developed an R package application, “NTAprioritization.R”, for fast prioritization of suspect lists, where candidates with potential exposure are prioritized based on their toxicity and identification evidence in the matrix. In this workflow, candidates were first rated for their identification levels based on spectral matching and retention time prediction. The level of toxicity levels was rated according to candidates’ toxicity of different endpoints or ToxPi score. Finally, the candidates’ level of exposure was ranked from Tier 1-5, in order of priority from highest to lowest. For validation of this workflow, it has been used to identify pollutants in a sludge water sample spiked with 28 environmental pollutants. The workflow reduced the number of potential candidates from 6,982 candidates to a final list of 2,779 compounds with 21 out of 28 spiked standards ranked in 5 tiers (Tier 1 to 5). Overall, this study shows the added value of an automated prioritization R package based on the NTA method for the fast screening of environmental pollutants.

### **3.15.P-We189 Assessing Pharmaceuticals In Red Seaweed through Ultra-High Performance Liquid Chromatography with Time-of-Flight Mass Spectrometry (UHPLC-TOF-MS) Multi-Residue Strategy**

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Red macroalgae have quickly become a valuable commodity worldwide, representing over 46% share of the major commercial seaweed market. Although their production is mainly directed for human consumption, many other purposes have been listed including food and feed industries, water treatment and remediation, medicine, cosmetics, and as biofuels (FAO, 2020). In 2018, *Gracilaria* species aquaculture accounted for over 3400 thousand tonnes, being the third most farmed genus. Another important use for these seaweeds is their role as bioindicators in ecosystem monitoring, given their proven ability to take up and bioaccumulate contaminants from the water and sediments. Therefore, it becomes paramount to have reliable analytical tools to their detection and quantification. Thus, the present work describes the development and validation of an ultra-high performance liquid chromatography with time-of-flight mass spectrometry (UHPLC-TOF-MS) multi-residue method for the quantification of 74 pharmaceuticals in *Gracilaria multipartita*. Since currently there is a gap on regulation and guidelines concerning the validation criteria specifically for seaweed, validation was performed following Commission Decision 2002/657/EC.

### **3.15.P-We190 Strategies for Monitoring Coastal Environments using Liquid Chromatography with High-Resolution Mass Spectrometry**

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Coastal environments present interesting challenges in environmental monitoring. Sewage outfalls, personal care products (PCPs), which may be partly attributed to the increasing popularity of open-water swimming, and the ubiquitous per-/polyfluorinated alkyl substances (PFAS) all contribute towards a cocktail of contaminants to which marine environments may be exposed. Challenges with sampling and the chemical composition of sea water present additional complications for analysis. Liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) is emerging as a key analytical technique in environmental monitoring, offering the benefits of non-targeted data acquisition and yielding data that can be flexibly interrogated by suspect screening and discovery workflows, as well as retrospectively analysed for novel contaminants of concern. This talk will present workflows for the analysis of emerging environmental contaminants in Irish coastal samples using liquid chromatography coupled with quadrupole-time of flight mass spectrometry (LC-QTOF-MS), covering experimental design, instrumental set-up and data handling.

### **3.15.P-We191 Identification of Tidal-Related Pollution Patterns in the Elbe River by Non-target Screening and Statistical Analysis**

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Tidal rivers are a vital and insufficiently studied nexus between freshwater and marine ecosystems where river-tide-interactions alter a river’s hydrology and geomorphology, but also the composition of the water. Constantly changing water level, flow rate and even flow direction can impact the behavior and fate of organic micropollutants (OMPs). By means of highly time-resolved sampling in combination with non-target screening and multivariate statistical methods, this study aimed at unravelling tidal-related pollution patterns in a large river and at identifying relevant sources and entry pathways.

In three sampling campaigns, hourly composite water samples were simultaneously collected at two monitoring stations. The stations were located along the tidal influenced section of the Elbe River, upstream and downstream of the urban area of Hamburg, Germany. Chemical analysis was performed by liquid chromatography coupled to an Orbitrap mass spectrometer. The software Compound Discoverer including an extensive in-house spectral library was used to perform peak picking, alignment, blank correction, and annotation of known OMPs. A self-programmed R workflow was employed to statistically analyze the resulting peak lists in order to unravel tide-related patterns.

Comparing annotated OMPs with water level data, up to now three different pollution patterns could be identified: a) features which are characterized by increasing intensities at the upstream station and decreasing intensities at the downstream station during high tide, and vice versa. Their main sources are obviously located between the two monitoring stations in the Hamburg area, b) features with increasing intensities during flood tide at both stations, supposedly introduced downstream of the Hamburg

area, and c) features with relatively constant intensities or patterns not related to water levels or direction of flow, likely originating from the upper reaches of the river.

This study confirmed that tidal dynamics substantially influence the behavior and composition of OMPs. Further, it was successfully shown that the applied sampling design allowed for an approximate localization of the sources. To gain a deeper understanding, monitoring is currently continued and the patterns are going to be analyzed further, i.e., by including additional physiochemical parameters in the statistical models. Moreover, unknown features in the dataset will be ranked and prioritized for subsequent identification.

### **3.15.P-We192 Dynamic Passive Sampling Methods for Marine Monitoring of Emerging Contaminants**

**Chloe Richards<sup>1</sup>**, Caroline Murphy<sup>2</sup>, Weili Guo<sup>3</sup>, Louis Free<sup>4</sup>, Sean Power<sup>4</sup> and Fiona Regan<sup>5</sup>, (1)School of Chemical Sciences, DCU Water Institute, Dublin City University, Ireland, (2)School of Biotechnology, Dublin City University, Ireland, (3)School of Biotechnology, DCU Water Institute, Dublin City University, Ireland, (4)Water Institute, DCU Water Institute, Dublin City University, Ireland, (5)Water Institute, Dublin City University, Ireland

Marine ecosystems are subject to a multitude of direct human pressures, such as overexploitation, eutrophication, pollution and species introductions<sup>1-3</sup>, including the effects of global impacts, namely ocean acidification and climate change<sup>4</sup>. These stressors can have synergistic effects on marine ecosystems<sup>5,6</sup>, altering their functioning and ability to provide goods and services<sup>7,8</sup>. Their impact is expected to be even stronger in enclosed and semi-enclosed basins with high population density, tourism flow and maritime activities<sup>9</sup>. Improved knowledge on the consequences of the effects of multiple stressors on marine biodiversity and ecosystem functioning is urgently required. The aim of this work is to develop a robust, sensitive and reliable method for the preconcentration of diclofenac in seawater using a dynamic passive sampling process.

### **3.15.V Innovations in Analytical Methods Used for Monitoring Emerging Contaminants in Marine and Freshwater Aquatic Environments**

#### **3.15.V-01 Quality Assessment of the Source Identification Applied to Different Spilled Oils and Performed by Distinct Methods**

**Ana Catarina Carvalho da Rocha<sup>1</sup>**, Carla Palma<sup>2</sup> and Ricardo Bettencourt da Silva<sup>3</sup>, (1)Division of Chemistry and Marine Pollution, Hydrographic Institute, Portugal, (2)Division of Chemistry and Pollution of the Marine Environment, Hydrographic Institute, Portugal, (3)Centre for Structural Chemistry - Institute of Molecular Sciences, University of Lisbon, Portugal

Chemical analysis performed on samples collected in the spill and suspected source(s) of its origin has been a valuable support for judicial investigations. The Gas Chromatography-Mass Spectrometry are used to obtain the sample fingerprint defined by a set of abundance ratios between chromatographic signals of specific components, i.e., diagnostic ratios (DR). The DR observed in two samples are compared using statistical methods and the equivalence between all DR in the set indicates sample composition equivalence, which allows identify the spill origin. The most common approaches to compare DR are based on Student's *t* statistics (S-t) (Nordtest method), and on a maximum relative difference of 14% (SC) (EN 15522-2 standard). However, the S-t approach assumes that the probability distributions of DR follow the normal distribution, while the 14% criterion relies on empirical knowledge that experts have acquired over the years, assuming a most expectable dispersion. Therefore, if inadequate assumptions or approximations are considered, erroneous assessments can be made about the equivalence of DR and fingerprints. The development of DR comparison approaches that better describe the reality of the variables under study is essential to ensure identification quality. This work compares the St and SC approaches with a developed alternative approach based on the accurate simulation of correlated chromatographic signals using the Monte Carlo Method. The study was carried out on International Round Robin test samples, made available by Oil Spill Identification Network of Experts, that represent spills of different petroleum products, allowing evaluate the impact of different DR sets on confidence intervals for DR comparison and on the probability (total risk) of true acceptance of compositional equivalence between samples.

#### **3.15.V-02 Assessment of Emerging Contaminants in an Anthropogenic-impacted Watershed: Application Using Targeted, Non-targeted, and In Vitro Bioassay Techniques**

**Theodora Lee<sup>1</sup>**, Caixia Li<sup>2</sup>, Mauricius Marques dos Santos<sup>3</sup>, Suan Yong Tan<sup>4</sup>, Mithusha Sureshkumar<sup>4</sup>, Khajornkiat Srinuansom<sup>5</sup>, Alan Ziegler<sup>5</sup> and Shane Allen Snyder<sup>4</sup>, (1)NEWRI, Singapore, Singapore, (2)Nanyang Environmental & Water Research Institute, Singapore, (3)Nanyang Environment & Water Research Institute (NEWRI), Nanyang Technological University, Singapore, (4)Nanyang Environmental & Water Research Institute, Singapore, (5)Faculty of Fisheries Technology & Aquatic Resources, Mae Jo University, Thailand

Monitoring the prevalence of emerging contaminants (ECs) in mixed-use watersheds is crucial for managing water resources effectively, particularly in light of growing population densities in cities worldwide. Restricted to a predefined set of target compounds, suspect screening by target analysis alone for water quality monitoring is insufficient to truly comprehend the impact of anthropogenic activities on aquatic ecosystems. Non-targeted screening (NTS) and biological risk assessments such as the use of *in vitro* bioassays (BSY), used in tandem with target screening (TS), are pivotal in ensuring a comprehensive assessment and data transferability to aid decision making processes in water quality management.

In this study, we investigated the presence of ECs in a mixed use Ping river catchment, Chiang Mai, Thailand, from remote, rural, agriculture, urban and subsurface sources from July 2021 to August 2022. The main objective of this study was to evaluate the use of three screening methods, TS using online solid phase extraction coupled to high-resolution liquid chromatography mass spectrometry (LC-MS/MS), NTS using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS) and *in vitro* BSY (cytotoxicity and reporter assays), to identify potential sources of pollution across a rural-urban gradient.

All three screening methods highlighted urban sites as the most polluted and toxic while remote sites were the cleanest and least toxic as expected. However, our NTS results further suggests that the contaminant profile across a rural to urban gradient is not simply linear, but the contamination profile of each land use type may vary for various reasons including the nature of contaminant use, and prevalent use of septic tanks in this region.

The results of this study also presented both the benefits and limitations of each screening technique, demonstrating how each technique can be used strategically in specific goal-oriented situations, or used in tandem synergistically to ensure high quality and well-informed water quality analysis.

### 3.15.V-03 Paper Microfluidic Device for Rapid Detection of Pathogens and Antimicrobial Resistance in Water

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Pathogen detection in wastewater is critical for early warning of pandemic, contributing to public health surveillance. We have demonstrated the application of paper-based microfluidic devices for veterinary diagnosis in India and malaria testing in Uganda, Africa. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes respiratory illness and gastrointestinal infections, with viral material being excreted in feces (and surviving within sewage for several days). Here, we propose a low-cost and user-friendly paper-based microfluidic device incorporating reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the detection of SARS-CoV-2 and influenza. Qualitative results were displayed by a UV torch, observed with naked eyes or recorded using a mobile phone camera. The paper-based platform could complete the concentration, extraction, amplification and detection of viruses in wastewater within 1.5 hours, with a detection limit as low as 10 copies  $\mu\text{L}^{-1}$ . The device was used for on-site detection of SARS-CoV-2 in wastewater samples from four quarantined hotels at London Heathrow Airport, showing results comparable to those obtained using reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays. The N gene presented the highest detection rate in wastewater samples, followed by the S and ORF1ab genes. ORF1ab gene shows less sensitivity than N, and S genes due to shorter sequence which is likely be degraded in the wastewater system. Our platform enables rapid detection of viruses without the need to send wastewater samples to centralized laboratories. Compared to gold-standard PCR assay, our platform provides similar or higher specificity and sensitivity for pathogen detection at a much cheaper and faster way, providing a high-resolution data set for highly responsive measurement during the pandemic. It has a clear potential that the platform can be used as a public health early warning tool for various applications in community settings and shows great potential for rapid and on-site wastewater surveillance at community settings in both developing and developed countries.

### 3.16.A Legacy, Emerging and Novel Per- and Polyfluoroalkyl Substances (PFASs): Latest Findings and Future Research Needs

#### 3.16.A.T-01 Polar and Nonpolar Interactions That Determine the Partition Properties of Neutral PFAS

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Per- and polyfluoroalkyl substances (PFAS) are a large group of substances with diverse molecular structures. To capture the emissions, transport, exposure, and risks of PFAS, their partition properties need to be understood. However, past studies were limited to a small number of PFAS of highest environmental concern. In this study, we measured isothermal gas-chromatographic (GC) retention times of 64 neutral PFAS on capillary columns with varying polarity to investigate the polar and nonpolar interaction properties of PFAS. The measured GC data were combined with partition coefficient data from the literature and our own lab and used to determine polyparameter linear free energy relationship (PP-LFER) solute descriptors, which quantitatively characterize the interaction properties of PFAS and can be used to predict various partition coefficients.

Retention data for the nonpolar columns were used to determine the log of the hexadecane/air partition coefficient ( $L$ ) as an indicator of nonpolar interactions, which was described in a previously published article. Measured log retention factors ( $k'$ ) obtained with the low polarity HP-5ms column exhibited a high correlation with  $L$  values, confirming the predominance of nonpolar interactions. Still, minor but significant difference was found in the trend of  $k'$  between nonpolar PFAS (e.g., perfluoroalkyl iodides, fluorotelomer olefins) and polar PFAS (e.g., fluorotelomer alcohols (FTOHs) and perfluoroalkane sulfonamides (PFASAs)). High polarity columns such as DB-225ms resulted in high scatter in the log  $k'$  vs  $L$  plot. ( $N$ -substituted) PFASAs deviated most strongly from the nonpolar PFAS baseline, indicating high polarity of these chemicals. FTOHs exhibited intermediate polarity, while fluorotelomer iodides, acrylates, and methacrylates demonstrated weak polarity.

PP-LFER solute descriptors (e.g.,  $S$ , dipolarity/polarizability parameter;  $A$ , H-bond donor property;  $B$ , H-bond acceptor property) were determined using the data for  $k'$  and partition coefficients ( $K$ ) and the calibrated PP-LFER equations. Preliminary results for FTOHs suggest that the  $A$  descriptor decreases with increasing  $-\text{CH}_2-$  spacer length. Also, it was indicated that  $N$ -substitution of PFASAs decreases the  $A$  property. These results are reasonable considering the structure of these PFAS. Currently, more  $k'$  and  $K$  data are being measured, which will yield new descriptor values and refine those that exist.

#### 3.16.A.T-02 Finding a Way Out? Biotransformation Study of Novel Fluorinated Surfactants

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Per- and polyfluorinated substances (PFAS) are a diverse group of anthropogenic chemicals with unique chemical and thermal stability. Stability made PFAS favorable for the industry, at the same time, caused concerns from the environmental point of view.

A new approach by the European Commission suggests a large-scale ban of PFAS and at the same time, highly supports the design of potentially safer alternatives. The goal of this study is to investigate the environmental fate of novel fluorinated surfactants designed with green chemistry sight.

Two trifluoromethoxy-substituted surfactants with either an ether or a thioether linkage were designed and synthesized. Microbial transformation studies under aerobic conditions in activated sludge-wastewater medium were performed for 126 days. A semi-closed experimental system with a trapping sorbent was selected to avoid any loss of volatile transformation products (TPs). The changes in the concentration of the initially added surfactants and the expected TPs were monitored by target analysis using liquid chromatography–tandem mass spectrometry. High resolution mass spectrometry (HRMS) was also used to identify other unquantifiable TPs.

**Results and Discussion:** Significant decrease in the concentration of the surfactants was observed over the incubation period. The main TPs were carboxylic acids (CAs), moreover, a shorter-chained CA representing the last product prior mineralization, was also detectable. Two additional TPs were identified by HRMS in the case of the thioether surfactant. The three CAs stayed quite stable during the incubation time and no final mineralization could be observed with our method. The total amount of the substances achieved only about 30-45% of the total expected concentration at the end of the incubation time. It was linked to the irreversible sorption of the surfactant molecules to the sludge.

Although mineralization of the surfactants was not observed based on the concentration monitoring of the TPs, it can be not excluded. When the use of any PFAS is essential, these prototype chemicals could be a compromise as their terminal products are less fluorinated and likely have less bioaccumulation potential compared to the conventionally used PFAS.

### 3.16.A.T-03 Development of Protein-Water and Membrane Water Partition Coefficients for Target and Suspect PFAS

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The unique chemical properties of per- and polyfluoroalkyl substances (PFASs) present a challenge for developing regulatory guidelines to protect organisms in the environment, and there is a need to establish protein-water and membrane-water partition coefficients to better characterize and model bioavailability in support of regulatory development. This poster/talk describes ongoing work to empirically-measure protein-water and membrane-water partition coefficients of 1) approximately fifty target PFAS using dialysis and the TRANSIL assay and 2) of approximately fifty target PFAS and additional suspect PFAS using biomimetic chromatography (BC) with detection by high resolution mass spectrometry (HRMS).

Membrane lipid-water partition coefficients ( $K_{mw}$ ) in variable pH for traditional and emerging PFAS were obtained by the TRANSIL Membrane Affinity assay. The  $K_{mw}$  for each PFAS is evaluated by the linear regression of log PFAS concentrations in the aqueous phase and the membrane lipid phase. The binding affinity of these PFAS for human serum albumin (HSA), the major carrier of PFAS in human blood, is also being evaluated and validated using equilibrium dialysis, the gold standard for protein-water partition coefficients. For these experiments, the rapid equilibrium dialysis (RED) assay provides a higher-throughput format using 96-well plates. Dissociation constants ( $K_D$ ) and PFAS-albumin partition coefficients ( $K_{PW}$ ) are evaluated by non-linear regression analysis.

Biomimetic chromatography has the benefit of directly simulating a biological environment using commercially available high-performance liquid chromatography (HPLC) columns containing immobilized artificial membrane (IAM) and human serum albumin (HSA) which act as bio-relevant stationary phases. Furthermore, protein binding of PFAS will be measured using HSA stationary phases whereas phospholipid binding will be measured using the IAM stationary phase<sup>1</sup>. The rapid-gradient approach allows for the determination of partition coefficients ( $k$ ) for a wide variety of compounds in a single, gradient-based HPLC run. Findings from the empirical measurements conducted at Oregon State University and University of Pittsburgh will be reported. Combination of the techniques will help further understanding of PFAS protein and membrane binding for future research needs.

### 3.16.A.T-04 Characterizing the Flow of Perfluoroalkyl Substances in an Avian Aquatic-Terrestrial Food Web

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Per- and polyfluoroalkyl substances (PFAS) are a group of emerging contaminants found ubiquitously in the environment. Limited research has been conducted on the flow of PFAS, including perfluoroalkyl sulfonates (PFASs), perfluoroalkyl carboxylates (PFCAs), their precursor compounds, and newer alternative PFAS, through aquatic and terrestrial food webs. As a species that forages on terrestrial and aquatic invertebrates, tree swallows (*Tachycineta bicolor*) potentially transfer and accumulate PFAS from both aquatic and terrestrial ecosystems. Our objectives were to assess the flow of PFAS in the aquatic-terrestrial food web of tree swallows, by: 1) determining food web structure using fatty acid (FA) signatures; 2) investigating PFAS patterns across abiotic and biotic food web compartments; 3) modelling PFAS contributions of food web compartments to PFAS burdens of tree swallows; 4) estimating bioaccumulation of target PFAS in this food web. We characterized 46 PFAS compounds in air, surface water, aquatic sediment, aquatic and terrestrial invertebrates, and gastrointestinal tracts (GIT) and livers of nestling tree swallows. The FA signatures of aquatic invertebrates had greater proportions of the FA 20:5n3, a FA typical of aquatic environs, vs. the terrestrial invertebrates, which had higher proportions of 18:2n6, a FA common to terrestrial biota. Tree swallow FAs were intermediate between aquatic and terrestrial invertebrates, although closer to the latter, consistent with aquatic and terrestrial feeding, but somewhat more terrestrial-based. Distinct patterns were evident in the PFAS profiles among ecosystem compartments: PFAS profiles of air, terrestrial invertebrates, and tree swallow tissues had higher proportions of PFOS than the aquatic compartments. The fluorotelomer carboxylate (FTCA) precursor, 5:3 FTCA, was a major component of the PFAS profiles

of aquatic sediment, aquatic invertebrates, and tree swallows (GIT, livers). Nonetheless, concentrations of most PFAS precursors were greater in aquatic samples than tree swallow tissues. Nestling tissues had greater concentrations of most long-chain PFASs and PFCAs than the other environmental compartments. These patterns are consistent with dietary uptake of PFAS by tree swallows via aquatic and terrestrial pathways and demonstrate the complexity of the flow of PFAS in this avian aquatic-terrestrial food web.

### 3.16.A.T-05 Multigenerational Toxicity of Per- And Polyfluoroalkyl Substances (PFAS) To *Daphnia Magna* and *Folsomia Candida*

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Per- and polyfluoroalkyl substances (PFAS) are a diverse group of man-made chemicals that have been widely applied and have raised environmental concerns across the globe due to their high persistence and ubiquitous presence in the environment. Short-chained PFAS have been used to replace long-chained PFAS, but they remain largely unregulated, such as perfluorobutanesulfonic acid (PFBS) and its precursor perfluorobutane sulfonamide (FBSA). These compounds have been detected in the environment near 3M Zwijndrecht, a PFAS hotspot in Belgium. The persistent and bio-accumulative features of these compounds call for chronic and multigeneration ecotoxicity tests, which are, however, virtually lacking. The aim of the present study was therefore to assess the multigenerational toxicity of PFBS and FBSA to the reproduction of an aquatic (*Daphnia magna*) and a soil-dwelling (*Folsomia candida*) arthropod species exposed for 6 and 5 generations, respectively. The toxicity tests followed OECD guidelines 211 and 232, respectively. The results of the single-generation tests showed that FBSA is much more toxic than PFBS to *D. magna* (EC<sub>50</sub>s of 5.48 and 856 mg L<sup>-1</sup>, respectively) and *F. candida* (EC<sub>50</sub>s of 1.21 and >1000 mg kg<sup>-1</sup> soil<sub>wd</sub>, respectively). Although the multigeneration toxicity tests with *F. candida* are still ongoing, we already showed that the EC<sub>50</sub> values of PFBS for the reproduction of *D. magna* decreased significantly from 856 to 376 mg L<sup>-1</sup> in the first four generations (F0 to F3), while the EC<sub>50</sub> value of FBSA decreased significantly from 5.48 to 1.19 mg L<sup>-1</sup> in the first three generations (F0 to F2). These results demonstrate an increasing sensitivity of *D. magna* towards continuous exposure to PFAS over multiple generations, which could be related to increasing internal exposure concentrations due to PFAS accumulation. Therefore, we plan to analyze the PFAS concentrations within *D. magna* and *F. candida* from the different generations. It is concluded that conventional single-generation toxicity tests underestimate long term PFAS toxicity.

### 3.16.B Legacy, Emerging and Novel Per- and Polyfluoroalkyl Substances (PFASs): Latest Findings and Future Research Needs

#### 3.16.B.T-01 Applications of Non-Targeted Analysis in PFAS Investigations

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Historically, industrial producers and users of legacy per- and polyfluorinated alkyl substances (PFAS) have been a major source of chemical contamination to nearby communities. Following the general phase-out of legacy PFAS such as PFOA/PFOS, it has been the domain of non-targeted analysis (NTA) to identify replacement PFAS species introduced by industry. Environmental and public health organizations have a significant interest in identifying emerging chemical contaminants such as PFAS due to concerns for potential widespread release and the persistence and bioaccumulation of PFAS species. As such, over the last several years the US Environmental Protection Agency's Office of Research and Development (USEPA ORD) has applied non-targeted analytical approaches alongside traditional targeted PFAS quantitation techniques to expand the coverage of PFAS information in uniquely impacted locations. USEPA partnerships with state and regional governments have examined novel PFAS classes including novel PFAS fluoroether species such as chlorinated perfluoropolyethers (ClPFPEs), polyfluorinated side products of polyfluorovinylidene (PVDF) and further compounds containing unique head group moieties and ether linkages; identified specific replacement chemicals used in applications such as metal plating and PFOS-free AFFF; identified the presence of these replacement PFAS in local media, including biota; and supported evaluation of the effectiveness of various PFAS remediation techniques on legacy and novel PFAS. This presentation will discuss case studies of recent work by USEPA ORD in NTA investigations of PFAS sources. Source examination reveals both intentionally added chemical species and production byproducts as common origins for emerging PFAS. Non-targeted interrogation of treatment approaches for emerging contaminants indicates that existing treatment technologies can be effective controls for many undescribed species. The talk will additionally discuss efforts to ensure the consistent application of NTA approaches in partner laboratories, reporting of NTA results to interested parties, and decision making using non-targeted analysis as a data source.

#### 3.16.B.T-02 Closing the Organofluorine Mass Balance in Marine Mammals with Suspect Screening and Machine Learning-Based Semi-Quantification

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A recent survey estimated that more than 6 million per and polyfluoroalkyl substances (PFAS) exist on the database PubChem, yet only a fraction of these substances are routinely monitored. This discrepancy has led to concerns that targeted analysis may underestimate the extent of PFAS contamination in the environment. To address this, fluorine mass balance experiments aim to quantify unidentified PFAS through a combination of targeted analysis and total fluorine-based measurements. High resolution

mass spectrometry-based non-target and suspect screening is vital for identification of novel organofluorine substances. However, assessing the contribution of newly identified substances towards the fluorine mass balance is hampered by the availability of analytical standards. To address this, machine learning models that predict ionisation efficiency (IE) in LC-ESI-HRMS can be useful for estimating concentrations.

In this work, liver from marine mammals collected from Greenland and Sweden were subjected to a fluorine mass balance and HRMS-based suspect screening. Thereafter, a machine learning-based semi-quantification approach was validated and applied to suspects in an effort to close the fluorine mass balance.

EOF concentrations were lowest in west Greenland dolphins, and were entirely accounted for by known PFAS ( $\Sigma$ PFAS of  $53 \pm 10$  ng F/g). In comparison, the highest EOF concentrations were observed in Swedish dolphins ( $1222 \pm 600$  ng F/g) with only 46% accounted for by known PFAS ( $411 \pm 148$  ng F/g). In wet Greenland Pilot whales, two individuals displayed a closed fluorine mass balance while three contained an average of 31% unknown EOF.

Suspect screening revealed several novel PFAS not captured by targeted analysis, which were confirmed at CL2. This included e.g. fluorotelomer sulfonic and carboxylic acids, perfluorinated sulfonamides, which collectively accounted for significant portions of the EOF following semi-quantification.

This study represents the first time machine learning-based semi quantification was applied to suspect PFAS in an effort to close the organofluorine mass balance. This *in silico* approach can be useful for estimating the contribution of newly identified PFAS towards overall PFAS exposure; and (2) identifying which standards should be prioritized for development.

### 3.16.B.T-03 Fluorine Mass-balance in Pooled Serum Samples from Northern Norwegian Men and Women Between 1986 and 2015

**Lara Cioni<sup>1,2</sup>, Merle Plassmann<sup>3</sup>, Jonathan Benskin<sup>3</sup>, Ana Carolina Coelho<sup>2</sup>, Therese Nøst<sup>2</sup>, Charlotta Rylander<sup>2</sup>, Vladimir Nikiforov<sup>1</sup>, Dorte Herzke<sup>1</sup> and Torkjel Sandanger<sup>2</sup>, (1)Norwegian Institute for Air Research (NILU), Norway, (2)UiT - The Arctic University of Norway, Norway, (3)Stockholm University, Sweden**

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals used in numerous industrial and consumer product applications. Due to their widespread use, PFAS are ubiquitous in the environment and humans and wildlife are exposed globally. While regulation of two of the most well-studied PFAS, PFOS and PFOA, has contributed to declining concentrations in human blood, production of other PFAS continues unabated. Considering that only ~1 % of the over 4700 PFAS on the global market are routinely monitored, there is increasing concern that human exposure to these chemicals might be underestimated.

In this study, human serum samples from the Tromsø (Norway) population, collected in 1986, 2007 and 2015, were pooled based on sampling year, sex, age and type 2-diabetes diagnosis. Each of the 46 pools contained 8-15 individuals and was analyzed for total fluorine (TF), extractable organic fluorine (EOF), total oxidizable precursors (TOP) and 54 target PFAS. The aim was to evaluate the concentrations of TF and EOF in human serum, the portion of unidentified EOF, the contribution of oxidizable precursors and the impact of sex and age on all these fractions.

TF concentrations ranged from <25 to 1330 ng F/mL and EOF concentrations ranged from 13.3 to 45.3 ng F/mL. No significant differences between sampling years were observed for TF while for EOF a significant decrease was observed between 1986 and 2007 (-1.20 ng F/ml) and no differences were observed between 2007 and 2015. There were no associations with sex and age for TF and EOF. In 25  $\mu$ L of pooled serum, 10 of the 54 target PFAS were detected: PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS, PFOS, FOSAA, Me-FOSAA, Et-FOSAA. The sum of target PFAS concentrations was lowest in 1986 and highest in 2007. Known PFAS accounted for 23-100% of the EOF. The unidentified EOF fraction was largest in 1986 (22-77 %; median: 46 %), significantly lower in 2007 (0-40 %, median: 11 %) compared to 1986 and significantly higher in 2015 (0-56 %; median: 37 %) compared to 2007. Women had significantly higher concentrations of unidentified EOF than men, opposite to what is observed for known legacy PFAS. Oxidizable precursors ranged from 0.00 to 1.85 ng/mL and accounted only for a small portion of the EOF (0-4%), meaning that additional analytical tools are required to close the organofluorine mass balance in human serum.

### 3.16.B.T-04 Dermal Bioaccessibility of Per- And Polyfluoroalkyl Substances (PFAS) From House Dust; Influence of Topically Applied Cosmetics

**Oddný Ragnarsdóttir, Mohamed Abou-Elwafa Abdallah and Stuart Harrad, University of Birmingham, United Kingdom**

Human exposure to per- and polyfluoroalkyl substances (PFAS) has been shown to negatively impact human health, e.g. by decreasing immune response after vaccinations. Their stability as well as their hydrophobic and lipophobic properties make PFAS suitable for use in a broad range of products, such as: textiles and personal care products. Many of these PFAS-containing products come in contact with the skin. Still, very little is known about the possible human exposure to PFAS via the dermal pathway.

This study investigates, *for the first time*, the dermal bioaccessibility of PFAS from household dust. Physiologically-based *in-vitro* tests were performed to evaluate the bioaccessibility of 17 PFAS from indoor dust to different synthetic sweat sebum mixtures (SSSM). Additionally, the impact of commonly used personal care products (foundation, sunscreen, moisturiser and deodorant) on PFAS bioaccessibility in the most physiologically-relevant SSSM composition (1:1 sweat:sebum) was examined.

The composition of the SSSM greatly impacted the bioaccessibility of the target compounds. PFAS bioaccessibility in the 1:1 sweat:sebum mixture ranged from 54-92% for perfluorocarboxylic acids (PFCAs) and 61-77% for perfluorosulfonic acids (PFSAs). Of the selected personal care products, sunscreen and moisturiser significantly decreased the sum PFCAs bioaccessibility. Similarly, foundation and moisturiser significantly decreased sum PFSAs bioaccessibility while the presence of deodorant significantly increased their bioaccessibility.

Finally, preliminary estimates of human exposure to PFAS via dermal contact with indoor dust were calculated based on our bioaccessibility data. These estimates show that for some PFAS (e.g. PFOS and PFOA), dermal uptake could contribute as much to an adult's overall daily exposure as other well-characterised pathways, such as drinking water and dust ingestion. While further research is needed to more accurately determine the human dermal uptake of PFAS, the current study shows that dermal exposure could contribute significantly to total human body burdens of PFAS and should not be neglected in PFAS risk assessment studies.

### 3.16.C Legacy, Emerging and Novel Per- and Polyfluoroalkyl Substances (PFASs): Latest Findings and Future Research Needs

#### 3.16.C.T-01 Enrichment and Emission of Perfluoroalkyl Acids on Nascent Sea Spray Aerosol from the Oceans

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The ubiquitous presence of perfluoroalkyl acids (PFAAs) in the environment has raised global concern. Oceans used to be considered as the final sink of PFAAs, where they would stay and be diluted into the deep ocean over decades. Recent laboratory studies demonstrated that PFAAs in seawater can be enriched hundreds of thousands of times on sea spray aerosols (SSA) and re-emitted from oceans to the atmosphere. Field evidence has shown that PFAAs transported on SSA significantly contributed to observed concentrations of PFAAs in air in coastal regions. However, the magnitudes of the PFAA emission via SSA from global oceans is not well quantified. One of the reasons is that it is uncertain whether the laboratory derived enrichment factor (EF) used in the estimation is environmentally relevant. In this study, field experiments were conducted along a north-south transect of the Atlantic Ocean to study the enrichment of PFAAs in SSA. Strong linear relationships were observed between the concentration of PFAAs in seawater and in supermicrometer SSA (both normalized to Na<sup>+</sup> concentration), which indicates the EF of PFAAs on supermicrometer SSA varied little along the cruise. The field-derived EFs were higher than previously observed in the laboratory, which may be due to the difference between artificial seawater and natural seawater. Based on the field-derived EFs, concentration of PFAAs in seawater measured in the field experiments and modelled global inorganic SSA production flux modeled by the Norwegian Earth System Model version 2 (NorESM2), we estimate that the emission of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) from the Atlantic and from the global oceans via SSA is comparable to or greater than estimates for the other known global emission sources. The spatial variation of the concentration of PFAAs in global oceans is likely a greater source of uncertainty than the variation in the EFs when estimating PFAAs emitted with SSA globally.

#### 3.16.C.T-02 Environmental Distribution and Bioaccumulation of Understudied PFAS Surrounding Two Fluoropolymer Manufacturing Sites in Italy and the United States

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With the phase-out of some per- and polyfluoroalkyl substances (PFAS) like perfluorooctanesulfonic acid (PFOS), replacement PFAS have been introduced in consumer and industrial applications across the globe. Little is known about the occurrence, biological accumulation, or potential impacts of some of these replacement PFAS, even though some of them have been in use for years or decades. Here we used tandem mass spectrometry (MS/MS) and high-resolution mass spectrometry (HRMS) to evaluate emerging PFAS in surface water, sediment, and fish tissue from the Delaware River (USA) and the Bormida River (Italy). These river systems are adjacent to similar fluoropolymer manufacturing activities, with known overlap of specific PFAS processing aids in production at both sites. Previous work in both regions has demonstrated the presence of understudied PFAS incorporating at least one chlorine atom and one or multiple ether linkages, called chloroperfluoropolyether carboxylates, or Cl-PFPECAs. Environmental samples from both locations demonstrate an abundance of Cl-PFPECAs in surface water, sediment, and fish from the sampling region, with the highest levels observed adjacent to and downstream from each suspected point source, respectively. Surface water concentrations of Cl-PFPECAs were on the same order of magnitude in both locations, with maximum sum concentrations totaling ~1780 ng/L in Italy and ~750 ng/L from the Delaware River in the USA. C6O4, a novel ether-based PFAS, was found abundantly in samples from Italy, but this compound was not apparent in any environmental matrix from the US. Bioaccumulation factors (BAF) were calculated for ten chlorinated homologs in fish tissue compared to surface water concentrations. BAF estimates from both Italy and the USA were in good agreement and suggest that the larger molecular weight Cl-PFPECAs demonstrate bioaccumulative behavior equal to or surpassing PFOS (log BAF ≈ 3-4), with calculated logBAFs ranging from approximately 2 – 7. This work highlights the continuing evolution of PFAS occurrence in the environment and underscores the importance of non-targeted methods to screen for PFAS beyond limited targeted lists. These data also suggest Cl-PFPECAs may be equally or substantially more bioaccumulative compared to legacy PFAS. This work also underscores the importance of international collaboration to advance our understanding of novel PFAS in the environment.

#### 3.16.C.T-03 The Decomposition and Emission Factors of a Wide Range of Per and Polyfluorinated Alkyl substances (PFAS) in the Dry Pyrolysis of Various Contaminated Organic Waste Fractions

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General treatment options for organic waste contaminated with poly- and perfluoroalkyl substances (PFAS) are currently limited to incineration, composting or landfilling, all leading to eventual emissions to the environment. Dry pyrolysis is emerging as a promising new waste handling alternative for contaminated organic wastes, as it recycles waste to biochar, condensate and syngas products that can have various useful applications. However, the fate of organic contaminants like PFAS during dry pyrolysis is still uncertain. In the present work, a mass balance for 56 different PFAS was established during the pyrolysis of sewage sludges, food waste reject, garden waste and waste timber. Pyrolysis was done in a medium-scale unit (500-800 °C) with syngas combustion. All wastes contained PFAS (56 to 3651 ng g<sup>-1</sup>), but pyrolysis led to a ≥98% removal. PFAS residuals (0.1-3.4 ng g<sup>-1</sup>) were detected in biochars produced at temperatures up to 750 °C. Loads of emitted PFAS increased with temperature in a range from 3 to 268 µg tonne<sup>-1</sup> of biochar produced and were mainly associated with aerosols. Emissions only accounted for <0.1% of the total mass of PFAS in the wastes. The presence and potential environmental impact of thermal degradation products are the main remaining uncertainties.

### **3.16.C.T-04 Forever legacies? Profiling Historical PFAS Contamination Sources and their Current Influence on a Groundwater Source for Drinking Water**

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A wide range of PFAS residues was studied in an aquifer affected by historical PFAS contamination from a landfill and military airport. Samples were taken at two monitoring and four pumping wells at different depths ranging from 33 to 147 m. Comparison of results with previous research from 2013 with a more limited range of PFAS, showed decreasing in the concentrations and migration of PFAS both in depth and in distance over decade.

Using the PFAS profile and branched isomer contribution pattern as a source characterization tool, the landfill was confirmed to contaminate the groundwater in both monitoring wells, while the former airport was found to be the potential source for contaminating the deep water in the first monitoring well. Only deeper sections of one of the monitoring wells were affected by the airport. Pumping wells used to produce drinking water were not yet affected by both PFAS sources, however a new and yet unidentified contamination source with a different PFSA profile and isomer pattern influenced one out of the four sampled pumping wells. This work shows the necessity of implementing screening of potential historical PFAS sources for identification and prevention of future contaminant migration in groundwater nearby drinking water extraction wells.

### **3.16.P-Tu209 Perfluoroalkyl Substances in the Atlantic and Southern Oceans: Forever Around Us?**

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Perfluoroalkyl Substances (PFAS) are persistent synthetic organic chemicals widely used either as surface-active chemicals in the industry, in household consumer products, and a myriad of other applications. The high-water solubility and extraordinary persistence of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and other perfluoroalkylsulfonates (PFSAs) and perfluorocarboxylates (PFCAs) results in their ubiquity in the oceans, but with very slow removal in the marine environment, with only one report of PFOS degradation in seawaters. Furthermore, individual PFAS show a wide range of physicochemical properties, thus their transport, and interactions with organic matter and oceanic sinks may show significant differences among them, and remain unexplored. Among all the compounds, PFOS and PFOA have been the most extensively studied as they have been determined to be abundant, ubiquitous, and some reports suggest decreasing concentrations. Oceans are a major sink and reservoir of PFAS, eventually driving the global occurrence and trends of PFAS. Therefore, the objective of this work was to assess the spatial and vertical distribution of PFAS by sampling and analyzing samples taken during latitudinal transects in the Atlantic Ocean (from 40N to 55S) and in the Southern Ocean (from 61S to 71S). On the other hand, concentrations for PFOA and PFOS will be contrasted with temporal datasets to assess their potential decrease in the environment.

This study shows how ubiquitous PFAS are, being found not only in the Atlantic but also in remote areas such as the Southern Ocean. PFAS surface concentrations ranged from 62 to 1425 pg L<sup>-1</sup> in the Atlantic transect, and from 13 to 516 pg L<sup>-1</sup> in the Southern Ocean.

No statistically significant differences were found between Atlantic and Southern Ocean surface samples, nor between samples from the surface and the depth of the chlorophyll maximum. Vertical profiles showed surface enrichment and depth depletion profiles, consistent with the introduction of these chemicals on the surface by rivers, wet/sea-spray deposition, and slow transport to deep waters. Compared to previous studies, we found variable temporal trends. Both biogeochemical processes and different regulations, can explain the different profiles of PFOS, PFOA and other PFAS. A number of transport and biogeochemical processes will be explored to provide further evidence of the controls on the occurrence of PFAS.

### **3.16.P-Tu210 Indoor Exposure to Per- And Polyfluoroalkyl Substances in the Faroe Islands**

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Due to their environmental ubiquity and toxicity to life, per- and polyfluoroalkyl substances (PFAS), have been classified as a



group of emerging persistent organic pollutants. Given that humans typically spend 90% of their time indoors, main sources of exposure to PFAS include indoor air and dust alongside food and water. Developing reliable detection tools to quantify PFAS present in indoor matrices and evaluating the significance of different exposure pathways are therefore critical to understanding the potential threat to human health. Passive air sampler tubes were deployed in 40 homes on the Faroe Islands and dust samples collected from the same homes. Several targeted PFAS were detected in the indoor matrices: Most notably, fluorotelomer alcohols (FTOHs), which are precursors to the more stable perfluorinated alkyl acids (PFAAs), were present in all 40 homes, while both volatile and ionic PFAS were present in dust. For example, 6:2 FTOH dominated with concentrations ranging from 0.1 to 2.6 ng/m<sup>3</sup> in air, and 17.6 to 94.3 ng/g in dust. Concentrations of ionic PFAS in dust were dominated by perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorodecanesulfonic acid (PFDS) with concentrations ranging from 0.5 to 14.3 ng/g, 0.5 to 29.0 ng/g, 0.3 to 24.2 ng/g, and 0.9 to 20.1 ng/g respectively. Concentrations in air and dust were compared to those found in the drinking water and traditional diet consumed by the Faroese population to estimate the overall exposure and assess the importance of the various pathways. Alternatively, reported concentrations for PFAAs in pilot whales, a traditional part of the Faroese diet, range from below detection limits to 7.4 ng/g wet weight. Yearly exposure to PFOS and perfluoroalkyl carboxylic acids (PFCAs) via air and dust pathways is estimated to be equivalent to 9g of pilot whale. Nevertheless, this research highlights the potential of indoor air and dust to be possible routes of exposure to PFAS, even in remote locations.

### 3.16.P Legacy, Emerging and Novel Per- and Polyfluoroalkyl Substances (PFASs): Latest Findings and Future Research Needs

#### 3.16.P-Tu211 FluoroMatch Flow and Visualizers are New Tools for Streamlined PFAS Annotation and Visualization

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Suspect and nontarget screening with liquid chromatography based high-resolution tandem mass spectrometry can be used for comprehensive characterization of PFAS. Many structure elucidation algorithms are focused on the six main chemical elements necessary for life, namely carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P), and sulfur (S). This means that they tend to perform poorly when confronted with anthropogenic compounds such as PFAS.

FluoroMatch Flow and Visualizer were designed as an open-source solution for PFAS Annotation that is freely available from [innovativeomics.com/software](https://innovativeomics.com/software). FluoroMatch Flow directly processes vendor files and includes a systematic scoring framework to communicate confidence for every feature, alongside reporting confidence levels via the Schymanski schema.

FluoroMatch automates file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on precursor and fragment masses, and annotation ranking. The software library contains ~7,000 PFAS fragmentation patterns based on rules derived from standards and literature, and the software automates a process to add more compounds.

To aid interpretation, we added a Visualizer tool to the FluoroMatch suite of software based on Microsoft PowerBI. It provides interactive mass defect plots, accurate mass vs. retention time plots, MS/MS fragmentation plots, and annotation tables. Selecting a feature in one graph will adjust what is displayed in other views. This interactive cross filtering allows simplified evaluation of a feature, PFAS series, or other groups of features.

Validating the percent coverage and accuracy of annotations in real-world samples was challenging due to the case of known unknowns and unknown-unknowns. In this experiment, we used all-ion fragmentation to estimate that FluoroMatch covered 71% of CF<sub>2</sub> containing PFAS compounds. Alternatively, with CF<sub>2</sub> normalized mass defect plots, we estimate 56% coverage of compounds with the remaining being false negatives.

#### 3.16.P-Tu212 Predictability of perfluoroalkylated substances (PFAS) in homegrown eggs using local environmental and biotic variables

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The housing of free-ranging chickens has gained worldwide popularity over recent years due to its economic, nutritional and ecological benefits. However, homegrown eggs in private backyards have been associated with elevated pollutant concentrations, including perfluoroalkylated substances (PFAS). Within this exposure context, very little is known about the contribution of local environmental and biotic variables in explaining potential variation of egg concentrations. While experimental studies have demonstrated that PFAS concentrations in the soil are largely influenced by soil characteristics, very little is known about its implications on the bioavailability of PFAS in laying hens. Furthermore, the contribution to the egg PFAS burden of potentially important biotic matrices, which may serve as feed source to laying hens, has not been evaluated to the best of our knowledge.

The main objectives of this study are to examine the (in)direct influence of multiple soil characteristics, soil and biota (vegetables, earthworms) concentrations on the egg PFAS concentrations in free-ranging laying hens and to evaluate the usefulness of empirical models to predict PFAS egg concentrations, taking into account these environmental and biotic variables.

Soil, egg, vegetable, worm and water samples were collected from 91 private gardens across Flanders. These matrices were analyzed for 32 target PFAS and the following soil characteristics were measured: total organic carbon (TOC) content, cation-exchange capacity (CEC), pH, electrical conductivity and clay content.

Results showed that egg concentrations of all PFAS were positively and negatively related with, respectively, soil concentrations and soil exchangeable potassium. For PFOS, clay content showed a strong and negative association with egg concentrations, while the reverse relationship was true for C11-C14 perfluoroalkyl carboxylates (PFCAs). Moreover, the effect size of clay

content increased with increasing chain length for the PFCAs. Apart from PFBA, the most suitable predictive and explanatory models were obtained with soil concentrations, clay content, pH and exchangeable potassium as sole predictors in the regression models ( $R^2$  range: 49.9 - 69.8).

Cross-validation of the final regression models indicated that egg concentrations could be predicted with good accuracy, especially for PFOS and PFNA. However, training the model with additional field data is necessary to validate the models' predictability.

### 3.16.P-Tu213 Analysis of PFAS in food and food packaging by targeted and non-targeted approaches

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Per- and polyfluoroalkyl substances (PFAS) are wide-spread, persistent environmental contaminants. Their grease repelling, non-stick properties make them ideal for food-contact materials. However, the same resistant qualities that are beneficial for industry have made PFAS a major concern for environmental contamination due to limited degradation. Because of their ubiquity, longer-chain PFAS have recently been banned in several countries, but alternatives continue to emerge despite a limited understanding of potential health risks. Environmental contamination has ultimately led to several reports of contaminated food, through irrigation or uptake in animals. Thus, monitoring food and food packaging for PFAS continues to be imperative for understanding human exposure. Analytical efforts on PFAS analysis have largely focused on water, but analysis of food samples, and consequently the development of efficient analytical protocols for food, has recently gained more attention. This study aimed to investigate PFAS in food and food packaging using targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) to monitor 33 PFAS and LC-high resolution mass spectrometry (LC-HRMS-Orbitrap) for non-targeted screening. We have developed and validated a simple and efficient analytical method for analysis of PFAS in fish, meat and eggs, which provided satisfactory recoveries in these foods with matrix effects below 20%. Using this method, several PFAS compounds were found in fish samples, including perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorotridecanoic acid (PFTrDA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeA), perfluorodecanesulfonic acid (PFDS), perfluorohexanesulfonic acid (PFHxS) and perfluoro-1-octanesulfonamide (FOSA) at low ng/g levels. Even fewer methods have been published for PFAS monitoring in food packaging, especially when coupled with non-targeted analysis. We have developed and evaluated an efficient sample extraction protocol for targeted and non-targeted analysis of PFAS in food packaging samples. Analysis of various food packaging samples of different geographical origin revealed frequent detection of PFHxS, perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorooctanesulfonic acid (PFOS) at ng/g levels. Food packaging for dry meats had the highest frequency of PFAS detection.

### 3.16.P-Tu214 Target and suspect screening of 4,777 per- and polyfluoroalkyl substances (PFAS) in river water, wastewater, groundwater and biota samples in the Danube River Basin

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Per- and polyfluoroalkyl substances (PFAS) have been scrutinized in the regulatory context as some of them are persistent, bioaccumulative, and toxic. In this study, the state-of-the-art computational and analytical tools were employed for the target and suspect screening of 4,777 PFAS in the Danube River Basin (DRB; 11 countries). Target screening covered 56 commercially available reference standards of PFAS. Suspect screening covered all the PFAS suspect lists submitted to the NORMAN Suspect List Exchange, including 4,777 PFAS in total. Chemical screening of the PFAS was performed in liquid chromatography – high resolution mass spectrometry using NORMAN Digital Sample Freezing Platform (DSFP). In total, 82 PFAS were detected in the studied 95 samples of river water, wastewater, groundwater, biota and sediments. Among them 10 PFAS were detected by target screening, 72 additional ones were revealed by suspect screening. Utilizing a quantitative structure-toxicity relationship (QSTR)-based approach, predicted no effect concentrations (PNECs) were derived for each PFAS and used for ecotoxicological risk assessment. Eighteen PFAS of environmental concern were prioritized in at least one environmental compartment. Seventeen of the 18 ranked PFAS are not monitored under the current legislative framework. The presence of PFAS across the DRB indicates a potentially widespread occurrence of PFAS in Europe, which requires systematic regulatory monitoring with special focus on the prioritized PFAS.

### 3.16.P-Tu215 The universe of fluorinated polymers and polymeric substances and potential environmental impacts and concerns

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Per-/Poly-fluoroalkyl substances (PFAS) are a diverse group of surface treatment chemicals, although less than 6 % of the estimated 4,730 PFAS are reported to be of global commercial relevance, and can be divided into non-polymeric and polymeric categories. PFAS have been produced and used for several decades including in electric and electronic devices, fire-fighting foams, and textiles. Glüge *et al.* recently concluded there are more than 200 use categories and sub-categories for more than 1400 individual PFAS. To date, research has primarily focused on the identity, life cycle, hazard, and environmental occurrence, monitoring, biotic exposure and health risks of non-polymeric PFAS particularly perfluoroalkylcarboxylic acids (PFCAs), perfluoroalkanesulfonic acids (PFSA), and some of their precursors. PFOS, PFHxS, PFOA and long-chain (>C7) and their related chemistries, have been increasingly phased-out of production and regulated in global jurisdictions. Those PFAS classified as polymeric can be further sub-divided into fluoropolymers, perfluoropolyethers (PFPEs) and side-chain fluorinated polymers (SCFPs), but environmental studies are far less prevalent than for non-polymeric PFAS. The present review summarizes

fluorinated polymers and polymeric substances and discusses the potential environmental concerns based on the current state of knowledge. Fluoropolymer producers are interested in having fluoropolymers treated as “polymers of low concern (PLC)”, due to their stability and seemingly low environmental impact; it is the use of fluorinated polymer processing aids that has caused widespread contamination at the production and manufacturing sites. Regardless, compared to many non-polymeric PFAS, fluoropolymers, PFPEs and SCFPs have received comparatively little attention from environmental scientists and regulators, despite their manifold industrial uses and high volumes. The current information demonstrates that there is already a considerable environmental burden of fluorinated polymers and polymeric substances and potential reservoir of more bioaccumulative degradation products. Concerted action is needed to address the dearth of environment-associated information and understanding on polymeric PFAS. Conducting research on those critical knowledge and data gaps that are most relevant for soundly regulating/managing polymeric PFAS and starting with reduction of emissions and release into the environment.

### 3.16.P-Tu216 Defluorination of 14C-Trifluoromethoxy Benzoic Acid in Soil

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During the last decades the introduction of CF<sub>3</sub> or CF<sub>2</sub> groups in the chemical structure of active ingredients (e.g. plant protection chemicals or pharmaceuticals) was found to be a very useful tool for increasing the target accessibility and thus the overall efficiency. Without the fluorinated group, the potency of molecules is often reduced (e.g. due to less cell membrane permeability) with the consequence that a much higher application or dose rate may be needed to reach similar protective or curative effects. Sometimes the molecule even becomes ineffective.

It is therefore of high interest if potentially degradable fluorinated groups (in the sense of being mineralized) can be introduced into active ingredients so that the high potency can be kept but without the risk of forming persistent "arrow head" substances (degradates).

HO-CF<sub>3</sub> was identified from the literature as a substance meeting the OECD PFAS definition, but that is also known to be hydrolytically unstable. The goal of this investigation was to confirm the potential degradability in soil of a model compound after the introduction of an O-CF<sub>3</sub> group, on the basis that the hypothetical “arrow head” is not a stable molecule.

Trifluoromethoxy benzoic acid was selected and radiolabelled directly at the CF<sub>3</sub>-group to be able to directly follow the fate of the fluorinated carbon atom during soil degradation.

Results from incubations of <sup>14</sup>C-trifluoromethoxy benzoic acid in three soil types according to OECD guideline 307 are presented, showing that the C-F bond is cleaved and the carbon atom finally oxidized to CO<sub>2</sub>.

After an incubation period of 28 days, trifluoromethoxy benzoic acid was completely degraded (<1% of total applied radioactivity recovered in soil extracts). For all three soils, the major degradation product was CO<sub>2</sub> accounting for 62 - 73% of the total applied radioactivity.

The presented results show that a single CF<sub>3</sub>-group does not per se make a molecule or its transformation products persistent.

### 3.16.P-Tu217 "Perfluoroalkyl substances (PFASs) in food webs: a state-of-the-art review and future research agenda"

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This review work aims to analyse the current literature knowledge on Perfluoroalkyl substances (PFASs), their presence in the environment and accumulation and behaviour in food webs. PFASs can be subdivided in short-chained and long-chain chemical structures, the latter received scientific attention in the last years, since they accumulate along trophic structures. The available literature focusing on PFASs dynamics in trophic networks was scrutinized using Publish or Perish software. Several PFAS congeners were compared and among them, congeners with higher bioaccumulation rate have been identified. The results of the collected studies are generally consistent, showing that long-chained PFASs have a higher rate of bioaccumulation and biomagnification in organisms along food chains. Above all, the research mainly covered polar and temperate regions, while little is known for tropical regions, on which different environmental factors might play a role in the kinetics of these substances along the food web. Most of the studies were aquatic (marine and freshwater), while only a minor aliquot were carried out in terrestrial systems, for which information on PFAS dynamics on the food web is limited. Within the PFAS congeners studies in the last decades, for some of them regulatory actions were taken, and replaced with some short-PFAS alternatives. Short-chain PFAS did not show bioaccumulation in aquatic ecosystems. Nevertheless, also for short-chained PFASs data from terrestrial environments are still negligible. In the future, systematic research about the effects of the new emerging short-chain PFAS on the environment and their bioaccumulation or biodilution in the food web must be addressed. It would be appropriate to include multiple trophic-interactions along the food-web, to compare literature, and to apply a meta-analytical approach to quantitatively verify biotic (e.g., species identity and trophic habits) and abiotic (e.g., temperature) effects on PFASs bioaccumulation patterns.

### 3.16.P-Tu218 Distribution and Bioaccumulation of Per- and Polyfluoroalkyl Substances in the Aquatic Environment: A Mesocosm Study on PFAS Uptake from Sediment by an Aquatic Plant

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Per- and Poly Fluorinated Substances (PFAS) are a widely used group of anthropogenic compounds, with a multitude of industrial and consumer applications. The widespread use of PFAS together with their persistence and bioaccumulation potential has led to global contamination of the abiotic and biotic environment. Nevertheless, their distribution across environmental compartments,

including the potential uptake by aquatic plants, remains largely understudied. The aim of the present study was therefore to investigate the distribution and bioaccumulation of PFAS in the aquatic environment. To this end, we analysed the PFAS uptake from sediment by an aquatic plant. Sediment cores originating from a heavily PFAS-contaminated pond (Blokkeerdijk, Antwerp) and a reference site (lake Gaasperplas, Amsterdam) were sampled. In Laboratory mesocosms, shoots from the rooting macrophyte *Myriophyllum spicatum* were planted and left to grow in these sediments, overlaid with Dutch Standard Water (DSW). At the start and the end of the 56 days experiment, the distribution and bioaccumulation of 10 PFAS with different molecular structures, including 3 isomer pairs (L-PFOS, Br-PFOS, L-PFOA, Br-PFOA, L-PFHxS, Br-PFHxS, PFBS, FBSA, 6:2 FTS, PFECHS) were determined in all matrices. All selected PFAS were detected in the contaminated sediment, while 6 out of 10 were also present in the reference sediment. All but one compounds were detected in the roots and shoots of the plants that grew on the contaminated sediment, while 5 PFAS were present in the roots and shoots of the plants that grew on the reference sediment. The calculated Biota-to-Sediment Accumulation Factors (BSAFs) for the 3 isomer pairs showed that linear isomers had a higher bioaccumulation potential from the sediment to the roots of the plants compared to the branched ones. It is concluded that PFAS are widely present in aquatic environments and that their bioaccumulation potential is driven by their molecular structure.

### 3.16.P-Tu219 Per- and Polyfluoroalkyl Substances Research Priorities for Environmental Risk Assessment

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Per- and polyfluorinated substances (PFAS) are a group of > 6000 ubiquitously applied persistent industrial chemicals. The field of PFAS research is developing rapidly, but suffers from substantial biases towards specific compounds, environmental compartments and organisms. The aim of this perspective was therefore to highlight current developments, and to identify knowledge gaps and subsequent research needs that would contribute to a comprehensive environmental risk assessment for PFAS. To this end, we consulted the open literature and databases, revealing that the environmental fate of PFAS is based on the analysis of less than 1.0% of the compounds that are categorized as PFAS. Moreover, soils and solid particulate matter (SPM) remain largely understudied. To determine the bioavailability of PFAS, the capacity of different passive sampler types to reliably take up the wide variety of bioavailable PFAS from the environment has yet to be determined. Furthermore, PFAS bioaccumulation and food web transfer studies focus on a very limited number of compounds and are biased towards aquatic biota. Fish are predominantly studied, with aquatic invertebrates and macrophytes being included less frequently. The available ecotoxicity data revealed that only a few PFAS have been well-studied for their environmental hazards, and that PFAS ecotoxicity data are strongly biased toward aquatic organisms, emphasizing the need to catch up in the terrestrial environment. Considering the persistency and bioaccumulation of PFAS, more chronic studies, but also multigeneration studies on ecologically relevant test species are needed. Finally, we identified the urgent need to unravel the relationship between sorption, bioaccumulation and toxicity on the one hand and molecular descriptors of PFAS chemical structures and physicochemical properties on the other. The present perspective ends by systematically listing the presently identified research needs to contribute to a comprehensive environmental risk assessment for PFAS.

### 3.16.P-Tu220 Chronic Toxicity of PFOS to Aquatic Macroinvertebrates

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Perfluorooctane sulfonic acid (PFOS) is of high concern because of its wide application in consumer products and its toxic effects on human and animal health. PFOS has been known to be extremely environmentally persistent, bioaccumulative toxic, and capable of long-distance transport. Previous studies assessed the acute effects of PFOS on the traditional endpoints mortality and immobilization for crustaceans while chronic effects on insects and crustaceans were assessed including also different endpoints like reproduction, body length, and growth. Previous studies have found that insects are more sensitive than crustaceans so we decided to perform not only chronic test using two model crustaceans macroinvertebrates (*Gammarus pulex* and *Asellus aquaticus*), but also evaluate insect species at least till their emergence. For this we also performed tests with *Cloeon dipterum* while tests with *Chaoborus obscuripes*, Zygotera and *Chironomus riparius* are currently running. During the chronic toxicity, mortality, immobilization, emergence, and food consumption were evaluated. Each species' juvenile was exposed to 5 different concentrations ranging from 0 to 1000 µg/L for all three test systems.

The NOEC mortality was 1000 µg/L for *G. pulex*. *C. dipterum* exhibited a greater sensitivity than *G. pulex* and *A. aquaticus*. *G. pulex* and *A. aquaticus* showed a 14 and 28 days control mortality of 11%. *C. dipterum* showed a 13% control mortality at the end of the experiment. The EC50 of *G. pulex* was 13,859 µg/L and 3,247 µg/L after 7 and 14 days, respectively. *A. aquaticus* showed no effects up to a concentration of 10,000 µg/L. The NOEC value of 100 µg/L based on mortality suggests that *C. dipterum* is among the most sensitive freshwater organisms tested. *C. dipterum* showed EC50 of 317 and 131 µg/L for 7 and 28 days, respectively. The present study indicates that insects are more sensitive than crustaceans, so the follow-up chronic tests using insect species are currently running to observe the effects of PFOS on the emergence of insects. Further experiments will be performed on the toxicokinetics with the same freshwater macroinvertebrates to evaluate the bioaccumulation and biomagnification of PFOS.

### 3.16.P-Tu221 Hydrogen Carrier Gas for GC/MS and GC/MS/MS analysis with a Novel EI Source

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Concerns around helium price and availability are increasing the demand for alternatives to this traditional mobile phase in gas chromatography mass spectrometry systems (GC/MS, GC/MS/MS). Hydrogen is the best alternative for GC/MS analysis as chromatographic resolution is superior when compared to alternatives, leading to faster analysis times. Hydrogen however is a reactive gas, hydrogenation and dechlorination reactions can and do occur in the mass spectrometer electron ionization (EI) source. Consequences when hydrogen is used with traditional ion sources are variances to mass spectral ion ratios, spectral infidelity, and peak tailing. Therefore, a novel EI source for GC/MS and GC/MS/MS was developed and optimized for use with hydrogen carrier gas.

### 3.16.P-Tu222 Development of Suitable Methods for PFAS Sample Preparation in Different Matrices

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Per- and polyfluorinated alkyl substances (PFAS) get into the environment during their manufacturing process and further also during their use and disposal. Analyzing these PFAS compounds is challenging and dedicated lab equipment helps to avoid blind values and standardizes the processes in sample preparation. Currently, there are existing SPE cartridge solutions for extractions of PFAS from drinking water. However, solid samples (especially soil samples) have often the ability to strongly bind neutral and long chain PFAS. Further, more demanding sample matrices, for instance, environmental and food/feed matrices are handled with a more elaborate dual SPE or a combination of SPE and dispersive clean-up to remove matrix interferences. In spite of the tedious process, all of them lack the ability to enrich some specific PFAS in a sufficient way e.g. neutral sulfonamides or long chain PFAS. The challenge is to develop a SPE solution that is not cost intensive and at the same time effective for the clean-up and enrichment for all types of PFAS analytes. Additionally, some of the PFAS analytes are volatile and an appropriate method for sample concentration is needed. Analyte extraction of solid samples via manual shaking was compared with a pressurized liquid extraction. For the enrichment and/or purification of PFAS compounds solid phase extraction (SPE) was applied. SPE cartridge solutions of different compositions were used. For the critical evaporation step a vacuum centrifuge with cold trap was used. The samples were subsequently analysed by LC-MS/MS. A pressurized liquid extraction method was successfully developed especially to extract neutral and long chain PFAS from soil. An important point in the development of the method was the establishment of the blind value free PLE system. SPE cartridge solutions of different compositions show high recoveries, low standard variations and reliable clean-up of PFAS analytes in different matrices. An evaporation method where no volatile PFAS compounds were lost has been successfully developed using a vacuum centrifuge in combination with a cold trap. The presented workflow shows a streamlined sample preparation process for the PFAS analysis, which can be used for different kinds of PFAS including neutral sulfonamides and long chain analytes in various matrices. Further, SPE cartridges with a superior performance for enrichment and clean-up of PFAS molecules from water, soil and food matrices were developed.

### 3.16.P-Tu223 AN ULTRA-HIGH SENSITIVITY ANALYSIS OF PFAS COMPOUNDS IN MULTIPLE WATER SOURCES

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PFAS compounds are ubiquitous in our environment due to overuse and their lack of breakdown, ensuring that this will be a challenge for decades to come. Therefore, it is imperative to provide rigorous and sensitive analytical testing to regulate these compounds and try to limit their possible effects on human health.

In December 2020, the European Parliament and Council of the European Union released a new directive that sets the limit of PFAS in drinking water to 0.5 µg/L for all PFAS compounds identified, and 0.1 µg/L for a subset of PFAS compounds that are particularly concerning for humans. The 0.5 µg/L limit applies to all PFAS compounds in total. This method is suitable for drinking water, surface water and groundwater.

Mixed standards were prepared at a ratio of 2.5 mL LC-MS water to 2 mL 50:50 acetonitrile/methanol +0.22% formic acid to construct a calibration curve between 0.2-1000 ng/L. Samples were prepared with 2.5 mL of seawater from the Irish Sea added to 2 mL of 50:50 acetonitrile/methanol + 0.22% formic acid solution prior to analysis.

Chromatographic separation was performed using a Phenomenex Luna Omega PS C18, 100 Å, 100 x 2.1 mm, 3 µm, and a Phenomenex Gemini C18, 110 Å, 100 x 2.0 mm, 3 µm delay column. The injection volume was 50 µL. Mobile phase A was 20 mM ammonium acetate in water and mobile phase B was methanol. The analysis was performed using a SCIEX 7500 system, operated in electrospray ionization in negative ion mode.

This analytical method presented shows ultra-high levels of sensitivity with LOQ values ranging between 0.2 ng/L and 2.0 ng/L for 26 relevant PFAS compounds analyzed in LC-MS grade, drinking, ground and surface water. In addition to the sensitivity of the method, excellent levels of precision, linearity and accuracy were achieved; all within typical validation criteria. Three water matrices were spiked at either 0.2 or 0.8 ng/L depending on their LOQs. From this, the detection limits were calculated between 0.06 ng/L to 1.12 ng/L in three different water matrices. With contamination being a challenge for successful PFAS analysis, recommended steps are documented in this method to reduce both contamination and interference.

Testing of surface and ground water is important to ensure that these water sources are not contaminated and that drinking water sources are not affected.

### 3.16.P-Tu224 Critical Insights in the detection of PFAS in our environment: A deeper look at various methods in water samples

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Per- and polyfluoroalkyl substances (PFAS) represent a diverse group of synthetic fluorinated organic compounds that have been produced and widely used in industrial applications and consumer products since the 1930s. Common applications where PFAS are used include surfactants, fire-fighting foams, nonstick cookware coatings, lubricants, and coatings for food packaging. Because of their wide use, testing is of great importance to mitigate potential risks to our health and the environment. The work presented will demonstrate various PFAS methods accommodating the different global regulations while at the same time offering high-performance solutions to optimize your laboratory workflows, e.g. sample preparation. Global regulations and sometimes their standardized methods require various sample preparation steps to reach the required detection limits. In the work presented we will show various regulation specific methods (EPA, ISO and EU) accommodating exactly this feat including offline SPE, direct injection and online SPE. We will delve into some key methods that are turnkey and ready to implement in various testing lab.

### **3.16.P-Tu225 Validation of a weak-anion exchange solid phase method for determination of extractable organic fluorine including trifluoroacetic acid in water samples**

*Zongzhe He, Ian Cousins, Jonathan Benskin and Merle Plassmann, Stockholm University, Sweden*

Per- and polyfluoroalkyl substances (PFAS) are widespread contaminants of the aquatic environment, including surface waters and groundwater. As these natural waters are important drinking water sources and drinking water treatment methods are ineffective for many PFAS, drinking water also contains PFAS. Given the large number and diversity of PFAS, there are growing concerns that targeted methods underestimate the extent of PFAS contamination in natural waters and drinking water. To address this concern, a growing number of studies have paired targeted analyses with determination of extractable organic fluorine (EOF) in an effort to capture substances that would be otherwise overlooked by targeted methods alone. However, when applied to water samples, such experiments require additional rinse steps to remove inorganic fluorine, which inadvertently removes ultra-short chain perfluoroalkyl acids such as trifluoroacetic acid (TFA). With this in mind, the present work aimed to develop a new extraction method for determination of extractable organic fluorine (EOF) as well as ~50 PFAS, including ultra-short chain substances, in different types of water samples. A suite of spike/recovery experiments involving fluoride and TFA were performed in order to determine exactly where each component eluted from the cartridge. Thereafter, careful adjustment of rinse and elution steps was performed to remove fluoride while maximizing retention of TFA. The performance of the method was benchmarked against two published methods. Overall, our developed method showed a satisfactory removal efficiency of high concentrations of fluoride with improved recovery of TFA.

### **3.16.P-Tu226 PFAS Analysis to Address the New EU Regulations for 24 Compounds**

*Day Powell, Agilent Technologies, Inc., United Kingdom*

Environmental challenges have never been greater, whether analyzing the purity of drinking water or contaminants in wastewater, measuring indoor air quality, responding to natural or man-made disasters, or identifying emerging contaminants. Environmental analysis must be done more reliably, more efficiently, and with even higher quality results than ever before. Agilent's solutions enable our customers to answer new questions at the leading edge of life science, diagnostics, and the applied markets. This poster details the latest Agilent analytical method to address the latest directive from the European Commission for 24 PFAS compounds in waters.

### **3.16.P-Tu227 Ultimate Sensitivity for the Detection of Per- and Polyfluorinated Alkyl Substances in Environmental Water Samples**

*Claudia Rathmann<sup>1</sup> and Janitha De Alwis<sup>2</sup>, (1)Waters Corporation, Germany, (2)Food and environment, Waters Corporation, United Kingdom*

Sensitivity limits are constantly being challenged with the continued evolution in regulations and guidelines for per- and polyfluorinated alkyl substances (PFAS). It is still unknown what the official regulations from the US EPA will become, but if they follow similar guidance to the European Drinking Water Directive, individual PFAS could be regulated around 1 ng/L (parts per trillion). Direct injection approaches have become popular for PFAS analysis since they reduce the sample preparation time, and more importantly limit the risk of introducing contamination. Generally, this approach requires large injection volumes of 30 µL or more. With the introduction of the enhanced sensitivity of a new tandem quadrupole, large volume injections are no longer necessary to reach the required sensitivity in the ng/L range.

To be successful with a direct injection approach for PFAS analysis, instrument sensitivity is extremely important. Initial evaluation was performed using solvent standards to understand instrument sensitivity. Peak area, peak height, and signal to noise were used to make this evaluation. Enhancements were experienced in all three parameters, indicating a true sensitivity increase without a detriment to signal to noise. Previous applications of the direct injection approach required a 30 µL injection volume, but with the increased sensitivity, injection volume was reduced to 10 µL. Even using the reduced injection volume, limit of detection (LOD), determined using a S:N of 3, for most compounds was near or below 1 ng/L.

Additionally, the mixed mode C18 AX column had excellent retention for the short chain PFAS, especially ≤ C5 chain length carboxylates that have poor retention on C18 columns. This also makes this chemistry a great candidate as an isolator column. For the C4 carboxylate (PFBA), contamination in the mobile phase was successfully resolved 8 minutes from the eluting analytical peak.

As studies on PFAS continue to advance, the analytical technology used to support research need to continue advancing as well. Improvements in both the analytical and isolator column technologies demonstrated in this work, as well as enhancements in negative ion sensitivity from the Xevo TQ Absolute Mass Spectrometer are helping to support ongoing efforts in PFAS analysis. This allows for easier, more robust, and accurate options as PFAS analysis continues into the future.

### 3.16.P-Tu228 Investigating the Impact of the Liquid Chromatography Method on NTA Identification of Short-Chain PFAS in Industrial Samples

**Jacqueline Bangma<sup>1</sup>, Mark Strynar<sup>1</sup>, James McCord<sup>1</sup> and Jeremy Koelmel<sup>2</sup>, (1)U.S. Environmental Protection Agency, (2)Yale University**

Per- and polyfluorinated substances (PFAS) are a class of thousands of unique chemicals containing carbon-fluorine bonds. Due to their widespread and decades-long usage, many PFAS are globally detectable in environmental media, wildlife, and humans. The most investigated PFAS to date are long-chain PFOS and PFOA. Yet, in recent decades, many applications of PFAS have transitioned to using shorter-chain replacement compounds. While some short-chain compounds have been identified (e.g., PFBA), emerging short-chain PFAS are still a concern and often require the use of high-resolution mass spectrometry (HRMS) and non-target analysis (NTA) techniques for identification. Many current reversed-phase liquid chromatography (LC) methods used to investigate PFAS result in short-chain PFAS eluting from columns within or close to the column's dead volume. This results in a variety of drawbacks including little to no chromatographic separation of isomers, ion suppression from matrix effects, and an increase in in-source artifacts. As the industrial production and application of short-chain PFAS are on the rise, it is important to optimize LC methods for the identification of emerging short-chain PFAS. In this study, industrially contaminated water samples from across the US were isolated using a weak anion exchange (WAX) extraction method and analyzed in triplicate with either a standard reversed-phase C18 column with a ramping gradient (25:75 aqueous: organic starting gradient) or a dual-phase column with an isocratic gradient (60:40 acetonitrile: methanol) in line with a high-resolution quadrupole time-of-flight instrument. Agilent Profinder was used for feature finding and FluoroMatch was used for visualization, grouping (via homologous series detection), annotation (via accurate mass and diagnostic fragment ion matching), and scoring. All data processing steps and FluoroMatch settings were maintained across experiments, and results were manually confirmed whenever possible. Results revealed that the dual-phase column resulted in increased retention of short chain compounds, improved compound separation, and improved identification of emerging short chain PFAS.

### 3.16.P-Tu229 Adaptation of large panels of Per- and Polyfluorinated Alkyl Substances (PFAS) for routine analysis in Drinking and Environmental Waters by Direct Injection Using UPLC-MS/MS

**Jenny Louise Davies<sup>1</sup>, Janitha De Abwis<sup>2</sup> and Claudia Rathmann<sup>3</sup>, (1)Waters Corporation, United Kingdom, (2)Food and environment, Waters Corporation, United Kingdom, (3)Waters Corporation, Germany**

Per- and Polyfluoroalkyl Substances (PFAS), are extremely persistent in the environment. Their production and use have resulted in severe contamination of soil, water and food. To protect public health, advisory and regulatory limits continue to be created and updated. Consequently, routine PFAS analysis has become challenging as not only low detection limits are required, but extensive compound coverage is a prerequisite nowadays.

The purpose of this work was to demonstrate a direct injection UPLC-MS/MS method for the ultra-low level determination of multiple PFAS compounds in drinking and environmental waters. The method performance study was completed on an ACQUITY Premier™ System with a Xevo TQ Absolute™ and UniSpray™ ion source. Samples were prepared by dilution with an acidified organic solution containing internal standards directly into an autosampler vial.

A method validation study was carried out on 2 common drinking water and 2 surface water matrices. The method performance was assessed using 3 spike levels at 1, 5, and 10 ng/L for all analytes, with 6 replicates at each level. Average method performance for trueness, repeatability, linearity, and sensitivity was assessed through inter and intra-laboratory studies. Improvements in both the analytical and isolator column technologies demonstrated in this work, as well as enhancements in negative ion sensitivity from the Xevo TQ Absolute Mass Spectrometer are helping to support ongoing efforts in PFAS analysis. This allows for easier, more robust, and accurate options as PFAS analysis continues into the future.

### 3.16.P-Tu230 How to investigate potential emission sources of PFAS in consumer product materials? Comparative extraction approaches and sum parameter analysis by combustion ion chromatography analysis

**Philipp Roesch, Christian Vogel, Ute Kalbe and Franz-Georg Simon, Division 4.3, Bundesanstalt für Materialforschung und –prüfung (BAM), Germany**

Besides classical per- and polyfluorinated alkylated substances (PFAS), side-chain fluorinated polymers (SFPs) are widely applied as efficient anti-wetting and anti-greasing coatings in various daily applied consumer products such as outdoor apparel, carpetry or paper-based food contact materials. Although the fluorinated sidechains are chemically bound to a base polymer, their release can be triggered by environmental influences or wear-off, thus contribute as significant sources of PFAS in the environment. Since only little knowledge of PFAS and SFP composition in consumer products is available, a comprehensive analytical approach might be beneficial.

Herein, we investigate the comparison between full combustion analysis, classical extraction methods and a hydrolysis-based extraction process for PFAS containing consumer product samples, resulting in the distinct sum parameter values total fluorine (TF), extractable organically bound fluorine (EOF) and hydrolyzable organically bound fluorine (HOF), determined via combustion ion chromatography (CIC). As reported previously, basic hydrolytic extraction can be applied to convert chemically bound PFAS into soluble precursors (e. g. as fluorotelomer alcohols). Consequently, a lot more sources of PFAS can be made accessible for analysis, while conventional methods can only comprise soluble PFAS with low chemical interaction within the sample material. Depending on the investigated consumer product sample, we found that the hydrolysis-based sum values (HOF) were larger compared to when conventional extraction protocols were applied. Differences in the measured sum parameter values might be attributed to the presence of SFC's at the material surface. Alongside, gas chromatography coupled mass spectrometry (GC-MS) analysis was applied to validate the hydrolysis reaction and formation of respective fluorinated reaction products. In

order to evaluate the fluorine mass balance of all extracted samples, our studies were complemented with targeted analysis investigation via liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) based on state-of-the-art extraction and measurement protocols.

Overall, we have shown that sum parameter analysis in combination with different extraction approaches allows a comprehensive investigation of PFAS-containing product materials and can be used to assess the PFAS content of a wide range of consumer products.

### **3.16.P-Tu231 Towards a systematic workflow for screening and identification of polymeric per- and polyfluoroalkyl substances (PFAS) in consumer products**

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Within the class of per- and polyfluoroalkyl substances (PFAS), fluorinated polymers — including side-chain fluorinated polymers, fluoropolymers and perfluoropolyethers — pose a significant analytical challenge. In this work we systematically compare the performance of a wide range of analytical methods for determination of polymeric PFAS in consumer products, including cookware, textiles, electronics equipment, and hygiene consumables. The methods included two portable and non-destructive screening techniques (Fourier-transform infrared spectroscopy (FTIR) and handheld laser induced breakdown spectroscopy (HH-LIBS)), two total fluorine approaches (particle-induced gamma-ray emission spectroscopy (PIGE) and combustion ion chromatography (CIC)), and an approach for structural elucidation (pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS)). Overall, FTIR displayed good potential as a rapid screening method for coatings of cookware while HH-LIBS was not sensitive enough to detect fluorine in any matrix. Quantification of total fluorine (TF) using PIGE and CIC demonstrated an overall good agreement between these two methods for most of the consumer products, with concentrations ranging from 70-550 000 ppm TF in cookware, 10-6100 ppm TF in textiles, 20-2100 ppm TF in electronic equipment and 10-630 000 ppm TF in hygiene consumables. The highest concentrations were observed in products containing polytetrafluoroethylene (PTFE). Finally, pyr-GC/MS was used to confirm that the fluorine was indeed organic in nature, and in some cases structural information about the substance was obtained. This is one of the few studies to quantify fluorine in a wide range of polymeric consumer products, and highlights key knowledge gaps and future directions for standardization of a systematic workflow for the screening and identification of PFAS in plastics.

### **3.16.P-Tu232 Understanding Side-Chain Fluorinated Polymers and Their Life Cycle**

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Per- and polyfluoroalkyl substances (PFASs) comprise a class of synthetic chemicals that have attracted much public attention since the early 2000s. Over time, research and risk management measures have expanded to a wide range of PFASs, but mostly focused on non-polymeric PFASs. To ensure the sound management of the entire class of PFASs, it is equally important to understand polymeric PFASs, which include side-chain fluorinated polymers (SCFPs), fluoropolymers and perfluoropolyethers. This poster presents a report by the OECD/UNEP Global PFC Group, synthesizing scientific and technical information on the life cycle of SCFPs, which are polymers with a non-fluorinated polymer backbone and with PFAS moieties on the side chains. The report starts with a comprehensive overview on the chemical identities of SCFPs that have been on the global market, including a non-exhaustive list of 103 SCFPs and 42 monomers. Then it includes life-cycle information on different SCFPs separated according to structural repeating units in the polymer backbone, specifically: acrylates and urethanes, oxetanes, silicones, and ethoxylates. For each group, the analysis focuses on the production and use of respective SCFPs, presence of other PFASs in the commercial formulations, degradation of SCFPs during use and end-of-life treatment, environmental releases of SCFPs, and other PFASs present in the commercial formulations. This is followed by a summary of critical knowledge and data gaps and options for a way forward. Compared to many non-polymeric PFAS, SCFPs and their known and unknown degradation products have received comparatively little attention from scientists and regulators, despite their manifold industrial uses and high volumes, their propensity to release non-polymeric PFAS, and their potential environmental and health impacts. Concerted action by all stakeholders is needed to address SCFPs in an efficient and effective manner, including making funding available and conducting research on those critical knowledge and data gaps that are most relevant for soundly regulating/managing SCFPs in different jurisdictions, building on the gaps identified in the report.

### **3.16.P-Tu233 Directly Fluorinated Containers as a Source of Perfluoroalkyl Carboxylic Acids**

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Direct fluorination of plastics is performed to impart chemical resistance via exposure of polyethylene to fluorine gas to produce a thin, fluorine-modified surface layer. Leaching experiments were performed on directly fluorinated containers under various conditions and with different matrices, including foodstuffs. The average sum of PFAS concentrations measured from extraction of the fluorinated containers alone was  $63.75 \pm 13.2$  ng/g plastic. 7-day leaching experiments of fluorinated containers with water, methanol, and acetone produced sum of PFAS concentrations that ranged from 0.99-66.92 ng/g plastic. Leaching experiments of fluorinated containers with food matrices produced sum of PFAS concentrations ranging from 2.74-5.50 ng/g plastic. For both solvent and food leaching experiments, a subset of samples was subjected to leaching at elevated temperatures, generating sum of PFAS concentrations up to 1,300% higher. In all experiments, short chain perfluoroalkyl carboxylic acids were detected in the highest frequencies and at the greatest concentrations, with analyte concentration decreasing as chain length increased. An



estimate for PFAS released from these containers into food was determined to be 0.35-3.23 ng/kg body weight per week, showing ingestion of food from these containers could be a significant source of PFAS exposure. Based on the large number of applications where directly fluorinated containers may be used, the observation of PFAS leaching into products suggest regulations on the use of fluorinated containers are warranted for where these products provide direct routes of human exposure to PFAS, and further studies should explore the fate and exposure pathways when fluorinated containers are disposed or recycled.

### 3.16.P-Tu234 How much and which PFAS are in North American Food Packaging?

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Considerable and well-deserved attention has been directed towards the use of PFAS in food contact materials because of the potential for direct human exposure and for indirect exposures through contamination of waste streams. In this work, we estimated that only ~2% of food contact materials in the U.S. and Canada have PFAS intentionally added to confer water- and grease-repellency. At this low intentional usage, and when accounting for an estimated 50% of food contact materials containing PFAS at “non-functional” levels, an estimated 10,000 and at least 11 tonnes per year of polymeric and non-polymeric PFAS move from production to end-of-life, respectively. Of this flow, we estimate that about 7000 tonnes/year of PFAS are landfilled or enter compost facilities and from there have the potential to enter the environment. Next, we analyzed samples of fast food contact materials from Canada. We found that of 42 samples, 43% contained elevated total fluorine concentrations measured via particle-induced gamma-ray emission spectroscopy. Of these samples, bagasse cardboard bowls consistently had the highest levels of total fluorine. The 8 contact materials with higher levels of total fluorine were found to contain 5-14 individual PFAS, dominated by 6:2 fluorotelomer methacrylate (FTMAc) and alcohol (FTOH). After hydrolysis, the concentration of 6:2 FTOH increased by an average of 220 times, and up to 33.7% of total fluorine was released from samples, indicating hydrolysis is a useful tool to assess the amount of unknown precursors. n-target analysis detected fluorotelomer carboxylic acids (FTCAs/FTUCAs) which is consistent with the use of side-chain fluoropolymers in food packaging materials, suggesting their continued use in the US and Canada, including in fast food packaging. Their use has the potential to release monomeric PFAS that are mobile in the environment and could be of toxicological concern. However, our estimates of low intentional use of PFAS to confer water- and grease-repellency in these materials suggests that the food packaging industry is moving away from this non-essential use of PFAS.

### 3.16.P-Tu235 Presence and concentrations of replacement fluorosurfactant processing aids and other PFAS in the air downwind of fluoropolymer production plants

**Joost Dalmijn<sup>1</sup>**, Ian Cousins<sup>2</sup>, Jonathan Benskin<sup>2</sup>, Matthew Salter<sup>2</sup> and Jana Johansson<sup>2</sup>, (1)Department of Environmental Science, Stockholm University, Sweden, (2)Stockholm University, Sweden

Fluoropolymer processing aid (FPA) emissions have contributed significantly to global environmental levels of per- and polyfluoroalkyl substances (PFAS) as fluoropolymer production plants act as a point source of these substances to water and air. With the phaseout of the FPAs based on salts of perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) in multiple countries, various replacement FPAs have been introduced that are based on the salts of perfluoroether carboxylic acids (PFECAs). Additionally, efforts have been made by industry to reduce the emissions of PFAS from fluoropolymer production to the environment. This study aims to investigate the presence and concentration of replacement FPAs and other PFAS in the air downwind of fluoropolymer production plants in the Netherlands and the United Kingdom and the impact of the installation of an emission abatement system during the study at the former site. The particulate phase is collected using high-volume air samplers equipped with quartz fibre filters and after extraction analysed using liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS). e dissociated FPAs hexafluoropropylene oxide dimer acid (HFPO-DA) and perfluoro-3,6-dioxaoctanoic acid (EEA) were found in samples taken downwind of the production sites in the Netherlands and UK respectively. These results imply that, although emissions have been reduced, these fluoropolymer production plants continue to act as a point source of atmospheric PFAS.

### 3.16.P-Tu236 Investigations of PFAS around industrial manufacturing sites

**Mark Strynar**, Jacqueline Bangma and James McCord, U.S. Environmental Protection Agency

PFAS have gone from environmental obscurity two decades ago, to one of the most common representations of environmental contaminants at present. A long use history from global sources is evident in the environmental literature. Compounding the issue is the cooccurrence of historical PFAS, with contemporary produced/emitted PFAS in collected samples. rgeted analysis of environmental samples with low resolution MS/MS is the method of choice for known contaminants. U rtunately, only a select species (<100) of 1000s of PFAS are sufficiently known and amenable to targeted analysis. A series of industrial site investigations within and outside of the United States at active production facilities have provided new insights into current and future PFAS. Therefore, high-resolution mass spectrometry and non-targeted analysis (NTA) methods are more appropriate for novel compound discovery. Parsing and annotation of 100s to 1000s of mass spectral features from these investigations is time consuming and quite often leads to PFAS of unknown or suspect identification. Appl ation of mass spectral feature alignment (*m/z*, retention time abundance) coupled with application of the aftermarket FluoroMatch software packet has helped to make recent advancement in many of these site investigations. Such M application can simultaneously rule out legacy PFAS not

requiring further exploration (e.g. M-H, CO<sub>2</sub> loss, gas phase dimers) while identifying features demanding further scrutiny (e.g. CF<sub>2</sub> Kendrick mass defect homologous series). Water samples collected near and distant from production sites aid in source apportionment. This talk will focus on the procedures used to identify suspected and novel PFAS at these facilities while offering insight into future PFAS for consideration on a broader scale. Contemporary production and use facility emissions are a strong indication of future environmental contaminants. Even in unknown compound identification, having knowledge of homologous series grouping (e.g. CF<sub>2</sub>, CF<sub>2</sub>O, CF<sub>2</sub>CH<sub>2</sub>, others) and characteristic negative mass defect alone help in sifting through scores of data needing more intensive analyst follow-up. Examples of unknown compounds within a known homologous series, and well as novel one-off and homologs will be presented. The natural progression of methods from novel compound discovery to robust targeted methods hinges on this critical identification first step and is often at industrial production and use facilities.

### 3.16.P-Tu237 Identification of PFAS Hotspots in German Rivers: Target Analysis vs. the Direct Total Oxidizable Precursor Assay

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The group of per- and polyfluoroalkyl substances (PFAS) comprises several thousand substances. The ever growing number is a major challenge for their chemical analysis. One analytical approach to overcome the complexity of PFAS are sum parameters, such as the total oxidizable precursor (TOP) assay. In this study, we conducted a spatial monitoring study with more than 210 suspended particulate matter (SPM) and sediment samples from rivers and lakes from Germany and the Netherlands to analyse spatial differences and identify contamination hotspots. All samples were analyzed by target analysis and a modified TOP assay ('direct' TOP (dTOP) assay, in which a small amount of sample is completely digested, converting previously unmeasurable precursors to measurable perfluorinated compounds.

The analysis revealed substantial differences between the different water bodies in both, the level and type of contamination. The ΣPFAS concentrations ranged from <0.5 to 53.1 µg/kg dry weight (dw) in the target analysis and from <1.0 to 336.8 µg/kg in the dTOP assay. The levels of perfluoroalkyl acids (PFAA) were substantially higher in the dTOP assay compared to the target analysis demonstrating the significant presence of unidentified precursors in the samples.

As a simplistic approach to identify hotspots of PFAS contamination the 90<sup>th</sup> percentiles (P90) for target analysis (P90<sub>Target</sub>: 7.11 µg/kg dw) and dTOP assay (P90<sub>dTOP</sub>: 67.6 µg/kg dw), respectively, were used as thresholds. Both methods identified 17 hotspots, but only five of the sampling sites were consistently identified as hotspots by both methods. Thus, the majority of hotspots identified with the dTOP assay was overlooked by the classical target analysis.

The results of this study demonstrate the ubiquitous burden of PFAS in German rivers especially by unknown precursors. Only some of the hotspots identified in this study were (publicly) known before. At many of the hotspots and other sampling sites, however, the source of the PFAS contamination remains unknown and must be elucidated in the future to prevent further discharge into the environment.

### 3.16.P-Tu238 Identification of Local Sources of Perfluorinated Compounds - Case Study in the River Vantaanjoki Region

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The Vantaanjoki River is the most urban waterbody in Finland; almost 10% of the Finnish population lives within its catchment area. The Vantaanjoki acts as a spare raw water resource and the riverside is an important recreation zone for local citizens.

Chemical quality of the surface water of the Vantaanjoki fails to fulfil the criteria set by the Water Framework Directive of the EU. This is caused by high concentrations of perfluorooctanesulfonic acid in fish. Also, the highest surface water concentrations of other per- and polyfluoroalkyl substances (PFASs) ever detected in Finland have been measured in samples from the Vantaanjoki. High concentrations may cause risk for both human health and the environment. One source of PFASs has been identified to be the Helsinki-Vantaa airport located on the downstream of the river, but high PFAS concentrations occur also at the upstream sampling sites. This alludes, that unknown sources of PFASs to the Vantaanjoki exist.

We approached the PFAS source identification by conducting a PFAS screening campaign for surface waters, municipal waste waters and storm waters. Based on produced data, PFAS loads to the Vantaanjoki from the tributaries and wastewater treatment plants (WWTP) were calculated. In that way, we could identify the regions, which produced highest absolute and proportional PFAS loads.

We found that WWTPs have substantial impact on PFAS concentration in river water. However, their share of the total PFAS load carried by the river decreased to the downstream indicating other notable PFAS sources within the drainage area. PFAS load estimations for sub-basins highlighted the role of the airport and industrial areas as sources of PFASs. Due to their high PFAS concentrations, stormwaters were also recognized as potential PFAS sources to the Vantaanjoki both directly and via WWTPs. The PFAS profile at the upstream of the river was remarkably different comparing to the downstream. At the upstream, perfluoroalkyl carboxylic acids were the dominant group, whereas perfluorosulfonic acids dominated at the downstream. There were also differences in PFAS profiles between tributaries. These results indicate qualitative difference in PFASs sources located in different parts of the drainage basin.

Our approach proved to be an efficient way to identify significant PFAS source areas for which more detailed examinations should be directed. It is also easy to reproduce at other areas, which are affected by PFAS pollution.

### 3.16.P-Tu239 Insight in PFAS concentration within the Netherlands

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Since the early 2000's Rijkswaterstaat (RWS), as part of the Ministry of Infrastructure and Watermanagement has measured over 30 PFAS in its surface waters.

The current abstract is the follow-up to an earlier work (Jonker, 2017), in which a data analysis was presented of PFAS concentrations in national waters, as measured between 2008 and 2016. The current abstract gives an update. Information on trends is provided.

PFAS concentrations and loads over the years and, in addition, current concentrations can be tested against the standards for PFOA and GenX that have since come into force. In addition, The current abstract will go into a modified set of substances and, in addition to concentrations in water concentrations in biota measured between 2017 and 2019 are also included in the data analysis. This makes it possible to test against the PFOS biota standard and to link water and biota and thus gain more insight into bioaccumulation of PFAS in Dutch waters.

PFAS are ubiquitous in national waters. Already since the beginning of measurements by RWS in 2008 they are found at all selected sampling locations in the water phase; also at locations at sea. The highest concentrations are generally found in the Scheldt and the Canal from Ghent to Terneuzen, but at the other inland sites the concentrations of specific PFAS are remarkably close to each other.

Some 10 PFAS have been detected in biota sampled in national waters between 2017 and 2019 at 15 locations, including locations in the open sea. Measured concentrations ranged from 0.1 to 140 µg/kg wet weight, with the highest concentrations measured in fish from the Western Scheldt, can be considered particularly PFAS-contaminated. At all sites, the relatively highest concentrations measured for PFOS, which are above the applicable standard for biota. The poster will visualize the various trends in the Netherlands.

### 3.16.P-Tu240 Occurrence of PFAS in sea-spray aerosol from the Dutch coast

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Per- and polyfluoroalkyl substances (PFASs) are a diverse group of persistent chemicals that are widely found in environmental samples. Recent studies have shown that sea-spray aerosol (SSA) may contribute to atmospheric transport and spreading of PFAS across the globe. SSA is a suspension of particles in air generated by bubble bursting at the interface between air and seawater, which has been shown to contain PFAS concentrations order of magnitude higher than those measured in seawater. In this study, we investigated the occurrence of PFAS in SSA in two coastal locations in the Netherlands (L1 and L2). SSA was collected using a high-volume air sampler (equipped with a PM10 head) during winter and summer in L1, and in spring in L2, respectively. Results indicated that both perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSA) occurred in air samples collected in winter and summer in L1. During winter sampling, also one precursor (i.e., 6:2 fluorotelomer sulfonic acid) and hexafluoropropylene oxide dimer acid (HFPO-DA) were measured. PFAS precursors were also detected by means of non-targeted screening analysis. Concentrations in samples collected in winter (0.04–4.95 pg/m<sup>3</sup>) were consistently higher than those measured in summer (0.04–1.37 pg/m<sup>3</sup>), suggesting that seasonal variation may be expected at this location. In L2, PFAS concentrations (0.04–0.64 pg/m<sup>3</sup>) were similar, but lower, than those measured in L1 in summer. A positive correlation between concentrations of PFAS and Na<sup>+</sup> (typically used as a tracer ion) in air samples indicated that SSA may be an important source of PFAS in the atmosphere. These results show that SSA may contribute to the transport of PFAS in the atmosphere and potentially act as a source of contamination in coastal areas.

### 3.16.P-Tu241 A study of the SOURCE-TO-SEA occurrence of Poly- and perfluoroalkyl substances (PFASs) of emerging concern in Ireland

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Poly- and perfluoroalkyl substances (PFASs) are a group of synthetic organic surfactants that have become a global concern because of the toxicity and widespread presence in the aquatic environment and organisms globally. In this study, a new analytical method has been developed and validated for the analysis of 15 perfluorinated compounds in different water matrices [1]: river water, drinking water and seawater. Water extraction was performed in anion exchange solid phase extraction cartridges, and extracts were analysed by liquid chromatography in tandem with mass spectrometry. Recoveries for target analytes were between 35–120%, depending on the water matrix. Method detection limits were in the range of 0.5–17 ng/L. The validated method was applied to the determination of perfluorinated compounds in water samples around Ireland. Eight compounds out of fifteen were detected at least in one sample. Measured concentrations were higher in river water than seawater, and drinking water had the lowest levels, although still detectable for a considerable amount of compounds. The most prevalent compounds were PFPeA, PFOA and PFHxA, present in all types of water, and they had the highest concentrations. This work will demonstrate the variability in chemical type in a sampling campaign from source to sea in the river Liffey, Dublin Ireland.

**3.16.P-Tu242 A Detailed Assessment of Per- and Polyfluoroalkyl Substances (PFAS) in San Francisco Bay Sport Fish**  
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As a class of thermally stable and non-reactive contaminants, per- and polyfluoroalkyl substances (PFAS) are widespread in the environment. The high persistence of PFAS, along with the bioaccumulation risks of some compounds, have raised concerns of adverse effects on human and wildlife health. Beginning in 2009, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has monitored PFAS in sport fish. At present, no human health or regulatory thresholds have yet been established for PFAS in California fish. Previous analyses of Bay sport fish have identified PFAS, particularly in the southern portion of the Bay, as persisting over time at levels exceeding thresholds that have been established by other US states for the development of consumption advisories.

To better understand spatial patterns and temporal trends of PFAS in San Francisco Bay sport fish, this study examined 58 archived skinless fillet composite samples from four fish species collected at 10 locations in three sampling rounds in 2009, 2014, and 2019. Fish species were selected based on a number of criteria, including species that are: popular for consumption, sensitive indicators of problems (accumulating relatively high concentrations of contaminants), widely distributed, representative of different exposure pathways (benthic versus pelagic), and included in past monitoring. Of 58 archived samples analyzed, PFAS were detected in all samples, with a total 17 of 40 PFAS observed. Perfluorooctanesulfonic acid (PFOS) was the dominant PFAS detected followed by perfluorooctanesulfonamide (PFOSA), similar to previous analyses of Bay sport fish. In general, perfluorooctanoic acid (PFOA), short-chain perfluoroalkyl substances (e.g., perfluorobutanoic acid or PFBA, perfluorobutanesulfonic acid or PFBS), most precursors, and newer PFAS (such as Gen X and ADONA) were rarely detected, with concentrations generally close to detection limits when found.

The expanded breadth of available data from this study provides the means for a comprehensive assessment of spatial variation in PFAS in Bay fish, and a robust baseline for evaluating long-term trends. Retrospective assessment of temporal trends is possible, though changes in analytical methods over time limit rigorous analysis. Overall, findings from this study will further inform PFAS management actions to protect the health of humans and aquatic life.

**3.16.P-Tu243 Per- and Polyfluoroalkyl Substances (PFAS) In Sunfish From North Carolina's Haw River and Jordan Lake**

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants gaining global attention due to their persistence, bioaccumulation, and toxicity. Use and disposal of PFAS-containing products, such as nonstick pans and firefighting foams, has ultimately led to human exposure through food and drinking water consumption. Thus, understanding PFAS accumulation in different matrices and geographical locations is critical for setting meaningful regulatory and advisory limits. However, to date there are no fish consumption advisories for PFAS in the state of North Carolina. In this study, we evaluated PFAS accumulation in fish fillets for fish caught in Jordan Lake and the Haw River. Because Jordan Lake is the primary drinking water source for 700,000 North Carolina residents and is fed by the Haw River which has known PFAS, its potential as a source for human dietary exposure from both the fish and water is high. Therefore, to perform this study, we characterized PFAS in fillets from 38 individual sunfish (genus *Lepomis*) caught at five different locations in this watershed. For the evaluations, the PFAS were extracted from the fillets and analyzed with nontargeted measurements combining liquid chromatography, ion mobility spectrometry, and mass spectrometry (LC-IMS-MS) separations. Data analysis was then performed using the open-source software Skyline and our in-house library containing over 100 PFAS. Our findings showcased a total of 22 different PFAS detected in the fillets with 21 unique PFAS in the most contaminated fish fillet and at least 8 PFAS in every fillet sample. Several legacy PFAS that had previously been phased out of production were detected in all samples, including perfluorocarboxylic acids with carbon chain lengths between 10 and 14 and linear and branched perfluorosulfonic acids of 8 and 10 carbons. Additionally, the fish upstream of Jordan Lake in the Haw River had more PFAS species (median = 16 analytes detected) and higher PFAS abundances than the fish from Jordan Lake (median = 10 analytes). Finally, the comparisons of PFAS abundances in fish and water also illustrated different PFAS accumulation in the two different matrices, suggesting that effective PFAS monitoring should include both fish and water sampling.

**3.16.P-Tu244 Target, non-target and suspect screening of Per-and PolyFluoroalkyl Substances (PFAS) in stranded dolphins, sea turtles and sharks (Tuscany coast, Mediterranean Sea)**

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In this study, we investigated the PFAS occurrence in tissues of striped dolphins (*Stenella coeruleoalba*), sea turtles (*Caretta caretta*) and different shark species (*Prionace glauca*, *Carcharhinus plumbeus* and *Isurus oxhyrinchus*) stranded or accidentally caught along Tuscany coast between 2020 and 2022.

Diverse PFAS were measured at high concentration in the tissues of dolphins (blood, brain, liver and muscle), whereas only PFOS was detected above detection limits in blood, liver and muscle samples of sea turtles and sharks. Liver tissue showed the highest PFAS levels, whereby PFOS was the dominant compound of the fingerprint. PFOS levels in dolphin liver ( $148 \pm 104$  ng/g ww; N = 23) were 10 fold higher than in the liver of the other marine species ( $1.12 \pm 1.09$  ng/g ww; N=9 and  $1.22$  ng/g ww; N=2 for sharks and sea turtles, respectively).

Suspect screening analysis allowed identifying 2 novel perfluorosulfonamides (FBSA(C4) and FHxSA(C6)) in all the analysed tissues of dolphin, as well as a series of n:3 fluorotelomer carboxylic acid (FTCA) in liver tissue only. Lacking of accumulation pattern and elevated levels of PFCA in the liver suggested a high level of metabolism of PFCA precursor compounds. This hypothesis was supported by the presence in liver of high levels of n:3 FTCA - stable intermediates of the metabolism of perfluorotelomers. In contrast, the striped dolphin probably lacks, totally or partially, the ability of transforming perfluorosulphonamides to PFSA within the liver. Our findings demonstrated that PFAA and perfluorosulphonamides accumulate in the marine cetacean species, whereas in sea turtles and sharks living in the same area accumulation does not occur. Metabolism in liver of fluorotelomer precursors seems to be the main source of PFCA in dolphins. Further studies should be necessary to disentangle if the main pathway of exposure to precursors is the breathing or the diet.

### 3.16.P-Tu245 Evaluation of per- and polyfluoroalkyl substances (PFAS) level in *Parus major* eggs from Nord Eastern Italy

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Per- and polyfluoroalkyl substances (PFAS) are anthropogenic pollutants globally distributed. PFAS have raised increasing concerns in recent decades due to their persistence in the environment and toxicity to human and wildlife, birds included. Although some PFAS are regulated, the presence of their precursors and alternatives in the environment requires frequent monitoring. Wild birds are considered efficient sentinels of environmental contamination and bird eggs have been proven to be a good monitoring tool for assessing the presence and exposure to PFAS.

The aim of this study was to evaluate the PFAS levels in great tit (*Parus major*) eggs from areas characterized by different anthropogenic impacts. Therefore, during 2021 and 2022, great tit eggs were sampled from nest boxes installed in agricultural, urban and wooded areas of Nord Eastern Italy far from known contaminated sites. Eggs were freeze-dried and the extraction was performed by sonication with ACN/water mixture enhanced by salting out and acidification; extracts were purified on HybridSPE-Phospholipid to remove matrix suppression effects by phospholipids. Legacy and emerging PFAS were analysed by ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-Q-Orbitrap). The results allow matching anthropogenic impacts and levels of contamination, as well as to determine anthropogenic background levels due to diffusive pollution.

### 3.16.P-Tu246 Unravelling the complexity of per- and polyfluoroalkyl substances (PFAS) contamination in marine organisms

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Per- and polyfluoroalkyl substances (PFAS) are a family of thousands of synthetic organofluorine compounds of high environmental concern. Some of these compounds have proven to be persistent, mobile, bioaccumulative and able to biomagnify. The stability and mobility of PFAS in waters make the oceans the largest environmental reservoir of PFAS and as a result, marine ecosystems are widely contaminated with these compounds. The combined evidence of their ubiquitous distribution and toxic properties has led to the ban of some of them (e.g. perfluorooctanesulfonic acid and long chain perfluorocarboxylic acids called PFOS and PFCA respectively). However, many alternative compounds and precursors of unknown environmental fate or effect on living organisms remain in use. By applying a non-specific method for PFAS analysis in biota (the Total Oxidizable Precursor or TOP assay), we evidenced that unknown PFCA precursors were by far the major contributors to the total PFAS burden of bivalves of the French coasts. Thus, beyond the ~20 PFAS traditionally studied and monitored in the environment, a large number of PFAS are overlooked. In this context, there is now a crucial need to identify the compounds responsible for this contamination. It is also necessary to further our knowledge of their environmental behavior, including bioaccumulation potential and toxicokinetics. The objective of this work is to go beyond the traditionally investigated PFAS in order to obtain a more exhaustive vision of the contamination of French coastlines by PFAS. Our specific objectives are i) to provide a definitive identification of these substances using a non-targeted approach, ii) to determine if they are accumulated by marine organisms from contrasting ecosystems, such as bivalves of the French coasts from a biomonitoring program, organisms from sea bass and sole trophic networks of the Seine estuary, and top predators such as marine mammals from the Bay of Biscay. Finally, the last objective is iii), to assess the biomagnification capacity of newly identified compounds from zooplankton to sea bass and sole. This presentation describes both the samples we investigate and the methods used to achieve the aforementioned objectives. A detailed example of the annotation of putative PFAS found in real samples is also provided.

### 3.16.P-Tu247 Narrowing Down the Identity of Extractable Organofluorine in Blubber

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Unlike many other persistent organic pollutants that accumulate in lipid rich tissues, most known per and polyfluoroalkyl substances (PFAS) accumulate in protein rich tissues such as liver and blood. The recent discovery of elevated concentrations of

unidentified extractable organofluorine (EOF) in killer whale blubber shows this rule might not be applicable to all PFAS. The aim of this study was to narrow down the possible nature of the unknown lipophilic organofluorine.

Analysis of a second Greenlandic killer whale confirmed the original findings from Schultes et al. (2020), which showed that >90% of unexplained EOF and very similar target PFAS profiles. Thereafter, extracts were subjected to the total oxidizable precursor (TOP) assay, which revealed low to negligible perfluoroalkyl carboxylic acid (PFCA) formation, indicating the organofluorine substance(s) were not PFAA-precursors. Solvent extraction using hexane improved extraction efficiency compared to acetonitrile, suggesting a neutral/non-polar organofluorine substance. This observation was further supported by a lack of sorption to strong anion and cation exchange resins. Overall, these experiments point towards a neutral/non-polar non-PFAA forming organofluorine substance.

Efforts to characterize the purified extracts by  $^{19}\text{F}$  NMR were unsuccessful, possibly due to the relatively high detection limits of this technique. A negative result might also indicate that the organofluorine is comprised of several neutral substances and not just one or a few substances in high concentration. Finally, a suspect screen for fluorinated liquid crystal monomers (LCMs) was carried out by GC-HRMS. While these substances display physical-chemical properties which align with those of our unidentified organofluorine, no fluorinated LCMs were detected from this analysis. Future work will apply a broader GC-HRMS-based non-targeted screening in an effort to further narrow down the identified of this unknown organofluorine.

### 3.16.P-Tu248 Detection and quantification of thyroid transporter interference in European water effluents using TTR-TRB CALUX

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Chemical pollution of ecosystems, partly and increasingly due to human activities, is intensifying in the present time. A potentially harmful and still not well understood group of chemicals for human and environmental health are per- and polyfluoroalkyl substances (PFAS), which represent a range of synthetic chemicals containing carbon-fluorine bonds. This highly stable bond makes PFASs difficult to degrade and easy to transport. PFAS contamination has already been detected in groundwaters, surface waters and soils. PFAS have been shown to bind to the thyroid transport protein transthyretin (TTR) and hence interact with the hormone system. Impairment of the binding of thyroid hormones to their plasma carrier proteins (TTR, TBG, albumin) by PFAS and related compounds may have implications for the availability of free T4 to thyroid cells. The concentration of free T4 in plasma is crucial for the maintenance of thyroid hormone homeostasis. Hence, binding defect of T4 to TTR is of particular importance because TTR is the only carrier protein capable of transporting T4 across the blood-brain barrier and the placental barrier and is therefore of particular significance.

The present study is incorporated in the NORMAN joint program activity on “sources related effluents”. It addresses the identification of chemical fingerprints and toxicity profiles in different source-related effluents across Europe to characterize and prioritize source-related footprints. The present study focuses on the detection and quantification of adverse effects potentially caused by PFAS compounds within water sample extracts via receptor mediated bioassays. A set of 105 samples of several sources such as industry, agricultural areas, or wastewater treatment effluents was analysed using two thyroid-related bioassays (TRB- and TTR-TRB CALUX). Resulting bioequivalent concentrations were linked to a chemical profile which has been revealed within the project. In this respect, 7 PFAS components were quantified in a variety of samples. First results showed cytotoxic effects as well as TRB-activation within the samples in environmentally relevant concentrations. The impact of the TTR binding on TRB-activity will be discussed as well as the potential identification of toxicity drivers. Furthermore, the study aims to enable a larger scale application and evaluation of TTR-TRB-CALUX for biomonitoring of surface water quality in future.

### 3.16.P-Tu249 Per- and Polyfluoroalkyl Substances (PFAS) in Soil and Wild Boar Samples from a PFAS Hot Spot Area – a Comparative Chemical Fingerprinting Exercise

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Per- and polyfluoroalkyl substances (PFAS) have been manufactured for decades for a broad range of technical uses in industrial processes and products. Intentional or non-intentional release of these highly persistent organic compounds resulted in diffuse contamination of the environment across the globe. Moreover, local point contaminations (PFAS hot spots) are identified in increasing numbers in industrialised countries. Large-scale PFAS hot spots might stretch over several kilometres and are typically located near the facilities of fluorochemical industry or sites where PFAS have been used in production processes or PFAS-containing waste was disposed-off. Soil, groundwater and surface water are often severely PFAS-contaminated in these areas and subsequent accumulation in biota and food-chain transfer is causing concern regarding long-term adverse effects on local human and wildlife populations. Against this background, a data analysis was conducted to explore the transfer of PFAS from contaminated soils into wild boar (*Sus scrofa* L.). This omnivorous terrestrial mammalian is known for intensely digging soils in

search for food, and consequently, soil ingestion by wild boar is rather high. Surface soil samples were collected from a well-described PFAS hot spot in south-western Germany and underwent extraction and subsequent chemical target analysis by a multi method covering 41 PFAS compounds. Similarly, PFAS contents of liver tissue samples taken from wild boar individuals that have been culled in the hot spot area were determined as an indicator of internal exposure. The presentation will highlight the results of a comparative chemical fingerprinting exercise, i.e. PFAS soil contamination patterns are compared to PFAS contamination patterns in wild boar liver tissue. The results will be discussed regarding their general significance, considering potential exposure pathways and the biology of wild boars as well as knowledge on the bio-accumulative behaviour of the PFAS compounds detected.

### **3.16.P-Tu250 Legacy perfluoroalkyl acids and their oxidizable precursors in plasma samples of Norwegian women**

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#### **1. Introduction**

Per- and polyfluoroalkyl substances (PFAS) are a large class of persistent chemicals extensively used in industrial and consumer products. Perfluoroalkyl acids (PFAA) are the most well studied PFAS family and have been recognized to pose risks to the health of humans. Exposure to PFAA may occur from the ingestion of food and drinks, but also upon the breakdown of its precursors, which might form stable PFAA. Nevertheless, limited data about these precursors is available, raising concerns about their contribution to the total PFAS burden. In this study, plasma samples from the participants of the Norwegian Women and Cancer (NOWAC) postgenome cohort were analyzed to estimate the concentrations of PFAA precursors and assess the associations between selected lifestyle and dietary factors and these precursors.

#### **2. Materials and Methods**

The present study included plasma samples from cancer-free women (n=302) from the Norwegian Women and Cancer (NOWAC) postgenome cohort, who completed questionnaires regarding their lifestyle, and a semi-quantitative food frequency questionnaire (FFQ). The samples were analyzed for 20 PFAS, including PFAA and known PFAA precursors, by using target analysis and an optimized TOP assay for human blood. Associations between post-TOP PFAA concentrations and selected lifestyle and dietary factors were assessed by logistic regression.

#### **3. Results and Discussion**

A total of seven PFAA were detected in more than 70% of the study participants in pre-TOP PFAS analysis. In post-TOP analysis, seven PFAA showed increased concentrations in at least 30% of the study participants, indicating the presence of oxidizable PFAA precursors within these samples. The biggest increases in concentrations were observed for PFHxA, br-PFOA, and PFDA. Few significant associations between the changes in PFAA concentrations post-TOP assay and lifestyle and dietary factors were observed. These associations were not consistent across the compounds, and considering the small increase in the PFAA concentrations, the statistical power of the analysis was limited.

#### **4. Conclusions**

More than 90% of the study participants had detectable amounts of PFAA precursors, and more than 30% of participants showed increased concentrations of seven PFAA after the TOP assay. Thus, estimating the oxidizable PFAA precursors is a complementary approach to estimate the total PFAS burden in humans.

### **3.16.P-Tu251 Effective Treatment Of PFAS Contaminated Waste And Process Water Using Coagulation And Flocculation – Implications For Soil Washing**

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High concentrations of per- and polyfluoroalkyl substances (PFAS) are found in different types of wastewaters, including landfill leachate, domestic and industrial wastewater and in process water from soil washing facilities. The treatment of wastewater commonly includes a coagulation and flocculation step, where particles and parts of dissolved substances are separated from the liquid phase. For an environmental sustainable use of soil washing as a remediation method the treatment and reuse of washing water is crucial.

To evaluate the effectiveness of different coagulants and flocculants, six products that are commercially available were tested for their PFAS reduction efficiency. They were selected to represent the range of typical coagulants used in soil washing and wastewater treatment facilities. They consisted of metalloxides, polyacrylamides, activated carbon products, as well as one material specially designed for PFAS removal. To obtain a wastewater that could be considered typical to that produced by soil washing, a silt-clay-water mixture, spiked with PFAS in concentrations typically found at Aqueous Film-Forming Foam (AFFF) contaminated sites was used.

Preliminary results show reduction efficiencies for standard flocculants as high as 50% for long chain PFAS and up to 30% for short chain PFAS. Maximum reduction efficiencies for long chain PFAS can be reached using a lower coagulant concentration than that needed to remove shorter chain PFAS. The use of specially designed flocculant, as expected, led to the highest reduction of PFAS. Reduction efficiencies of long chain PFAS exceeded 98% and for short chain PFAS the reduction was over 85%.

The results of this study indicate that by choosing the right coagulant and flocculant in the wastewater treatment process, PFAS can be substantially removed from the water phase. This may facilitate further clean-up steps in a treatment train, such as increasing the life of activated carbon filters or reverse osmosis filters downstream of where the flocculation occurred. This is

important as the change of a coagulant or flocculant can be made while operating these facilities and without bigger investments in additional treatment infrastructure, which makes this approach fast and easy to implement.

### 3.16.P-Tu252 Removal Of Persistent And Mobile Perfluoroalkylated Substances (C1-C13 PFAS) From Real Water Matrices Using A Boron-Doped Diamond Electrode

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Poly- and per-fluoroalkyl substances (PFAS) are persistent organic chemicals that affect both aquatic organisms and humans. Hence, the European Union (EU) limited the presence of 20 PFAS (C4-C13) in water intended for human consumption by the 2020/2184/EU Directive. Besides, the recent proposal of revision of the EU Water Framework Directive (Directive 2008/105/EC) proposed to establish environmental quality standards for up to 24 PFAS.

Given the fact that conventional (and several advanced) treatments are ineffective in removing PFAS, new technologies shall be developed to cope with this global threat. The present work aimed at assessing the performance of the anodic oxidation (AO) process using a boron-doped diamond (BDD) anode for the removal of PFAS from different aqueous matrices. Both the several regulated PFAS (C4-C13) and the ultrashort-chain, very mobile, PFAS (C1-C3) were investigated (24 carboxylates and sulfonates, in total) at realistic concentrations (down to 0.2 µg/L). The effect of the five following matrixes was assessed: ultrapure water, drinking water, urban wastewater after secondary treatment, reverse osmosis and nanofiltration concentrates from urban wastewater tertiary treatment. Experiments were carried out at different current densities, either in the presence of a mixture or just one single PFAS. PFAS with carboxylic moieties proved to be more easily degraded than those with sulfonic ones. Whereas the former PFAS were (almost) completely removed, the removal of the latter ones greatly varied with the carbon-chain length, from less than 10% for C1 to complete (<LOD) removal from C8 on. The presence of other species in real matrices affected the efficiency of PFAS removal, but not hindered the use of the AO process.

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### 3.16.P-Tu253 Pilot testing and economic analysis of PFAS removal from groundwater using granulated activated carbon and Sorbix™ ion exchange resin at a Swedish airport

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Skövde airport is located in south-central Sweden. It was inaugurated in 1989 and was used for regional commercial flights from the early 1990's until the early 2000's. A series of environmental studies revealed substantial PFAS contamination in surface soils, surface water and groundwater. The municipality engaged ECT2 to run pilot tests to evaluate the most economically beneficial technology to remediate the groundwater at the site. PF concentrations in the groundwater were shown to be as high as 17 000 ng/L for the sum of eleven PFAS species that are used in Sweden to assess PFAS contamination. These pilot tests were performed in 2022 and compared granulated activated carbon with ion exchange resin. The aim of the remediation is to bring PFAS levels down to under 90 ng/L for the sum of eleven PFAS. The pilot testing showed that the ion exchange resin was more than twelve times more effective on a per volume basis at treating the groundwater to under 90 ng/L and three times less expensive than a GAC solution.

### 3.16.P-Tu254 Life CAPTURE, a Project for the Sustainable Management of Contaminated Sites by Per- and Polyfluoroalkyl Substances (PFAS)

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LIFE CAPTURE is a project that has been funded by the European LIFE ENVIRONMENT programme to the tune of approximately EUR 5 million. The project is coordinated by ABO and involves 8 European institutions including research institutes and public and private companies.

The overall objective of LIFE CAPTURE is to develop sustainable management methods for the treatment of per- and polyfluoroalkyl substances (PFAS) in soil and groundwater.

The aim is to develop a robust protocol for the analysis of PFAS, which is currently lacking, and to broaden the spectrum of the PFAS family that can be analysed. LIFE CAPTURE proposes a robust protocol that follows a step-by-step approach: to quantify the presence of different types of PFASs; to quantify the concentrations of a predetermined group of well-studied PFASs; to determine whether other PFASs are present; and to quantify and qualify their concentrations.

During the investigation of a contaminated site, it is currently considered good practice to only observe concentrations. The use of flow measurements would be a valuable addition, allowing a more direct measurement of exposure and risk. It can also be used to drastically improve the efficiency of mitigation measures such as remediation. In this project, passive flow samplers will be used, which can be integrated into the new analytical protocol.

Due to the chemical properties of PFAS, most existing remediation technologies do not provide adequate sustainable treatment



solutions. We propose a toolkit of promising innovative remediation technologies for PFAS (e.g. foam fractionation for soil treatment and AOP for groundwater remediation). Technology trains will be identified and tested at the laboratory level to optimise remediation approaches to remove contaminants from the environment. The technology trains will then be tested in representative environments through pilot tests at four contaminated sites in Belgium and Italy. The assessment of whether a given PFAS contamination is harmful is generally governed by a regulatory framework. It is proposed to develop a pragmatic approach to risk and effect assessment. This will be based on existing standardised ecotoxicity tests. Particular attention will be given to risk assessment for mixtures of different known or unknown PFAS.

### **3.16.P-Tu255 Connecting the Dots: PFAS Risk Framework and Communication Hub**

**Mark Ballentine**, David W. Moore, Alan Kennedy, Manoj Shukla, Bill Gardiner and Jodi Ryder, *U.S. Army Corps of Engineers*  
Per- and Polyfluoroalkyl substances (PFAS) in the environment continues to evolve and grow in complexity with no obvious risk management strategy to protect citizens worldwide. The growing number of PFAS compounds (more than 10,000) and the ever-changing and large amount of scientific data being generated increases risk-management uncertainty; this uncertainty slows timely, science-based risk-informed decision-making for military installations and site managers. The U.S. Army Engineer Research and Development Center is developing a science-based, rapid risk characterization framework for PFAS across a broad range of environmental media (water, soils, sediments) to enable better informed and cost-effective management solutions. The framework and communication hub will connect users to requisite resources and subject matter experts facilitating more timely decisions. Experimental case studies (from micro- to mesocosm) are planned to bridge the gap between high fidelity laboratory generated data and field observations improving understanding of key mechanistic drivers required for derivation of risk estimates and reduce uncertainty. The data generated will be utilized in computational models to characterize fate and effect of PFAS in the environment. These computational models will ultimately be used to optimize an existing multi-media fate and transport modeling framework for field scale PFAS concentration screening. The multi-year effort's goal is to create a working PFAS risk framework prototype and communication hub to direct users to needed scientific data, tools, and standards.

### **3.16.P-Tu256 Luminescence Lifetime-Based Sensing Platform Based on Cyclometalated Iridium (III) Complexes for the Detection of Perfluorooctanoic Acid in Aqueous Samples**

**Kun Zhang**, Stuart Harrad Prof and Zoe Pikramenou, *University of Birmingham, United Kingdom*

The EU has listed perfluorooctanoic acid (PFOA) as a substance of very high concern. Given the high cost and time-consuming nature of current LC-MS methods for measuring PFOA and other perfluoroalkyl carboxylic acids (PFCAs); less expensive, rapid methods for the quantification of PFCAs and other perfluoroalkyl substances (PFAS) are urgently required. Luminescence lifetime is an attractive analytical technique for detection due to its high sensitivity and window of detection which varies based on the choice of the luminescent probe. Iridium probes exhibit luminescence with long lifetimes which are sensitive to the local environment. Herein, a novel and facile lifetime-based luminescence sensor for PFOA was prepared based on gold surfaces modified with lipophilic iridium complexes bearing alkyl chains, namely, IrC6bpySS and IrC12bpySS. Upon addition of PFOA, the luminescence lifetime of the iridium complex decreases allowing monitoring of PFOA in aqueous solutions. The platform was tested for measurement of PFOA in drinking water samples spiked with known concentrations of PFOA and demonstrated determination of PFOA for concentrations >100 µg/L (240 nM).

### **3.17 Measuring, Modelling, and Monitoring the Environmental Fate and Exposure of Pesticides**

#### **3.17.T-01 Determining the Fraction of Global Freshwater Ecosystem Carrying Capacities Exceeded by Pesticide Use**

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Pesticide use nearly doubled from 1990 to 2018, and is projected to increase further with population growth. To determine whether current pesticide use practices exceed the limits for an ecosystem to withstand pesticide pressure, protective thresholds for environmental compartments must be identified across ecosystems at relevant spatial scales. Therefore, methods are needed to both calculate and link the actual pesticide pressure on ecosystems (as a result of pesticide usage) and the capacity for ecosystems to withstand that pressure. Challenges in developing these methods include variability in where pesticides are used, and spatial differences in features of ecosystems (e.g., climate, location of water bodies). Additionally, regions with seemingly distinct carrying capacities may be interconnected as pesticides emitted into one region may occupy the carrying capacity in other (e.g., downstream) regions. To address this gap, we introduce a spatially explicit framework to relate pesticide pressure to region-specific carrying capacities. We then assess current pesticide use against freshwater ecosystem boundaries globally and quantify the fraction of freshwater ecosystem carrying capacities that is occupied (and potentially exceeded) by toxicity-related pressure from pesticides. Pesticide application data for 865 chemicals was translated to emission mass to air, soil, and water. These data were integrated into the Pangea spatial multi-scale model to determine the concentration of each chemical in 87,063 freshwater catchments globally. Pesticide pressure and carrying capacity were combined, so the output for each chemical in a region is the fraction of a freshwater ecosystem's carrying capacity occupied by that pesticide,  $F_{cc,o}$  (considering both direct and indirect contributions into that region). For each region, the  $F_{cc,o}$  was analysed per pesticide and cumulatively (across pesticides). We found that most pesticides do not exceed any carrying capacities individually, but some frequently used pesticides do in a subset of catchments (e.g., atrazine and imidacloprid in the eastern United States). Further, up to 15% of global freshwater ecosystems have cumulative pesticide pressure exceeding their carrying capacities (based on the sum of individual pesticide  $F_{cc,o}$  per catchment). This framework can help identify chemicals dominating cumulative chemical pressure in order to guide environmental remediation and inform more sustainable pesticide use practices.

### 3.17.T-02 Leveraging SWAT+' New Landscape Routing, Conditional Management, and Pesticide Functionalities

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The Soil and Water Assessment Tool (SWAT) has been an internationally leading model for predicting the fate and transport of non-point source agrochemicals at the watershed scale for nearly two decades. SWAT is a semi-distributed model based on a hydrologic response unit (HRU), a conceptual component that aggregates areas with similar landscape characteristics within a watershed, including land use and soils, to simplify the calculation of water fluxes, as well as sediment and chemical loading to a channel system. Strengths of SWAT for the simulation of agrochemicals include the comprehensive hydrologic model, channel routing and in-stream chemical transport processes, and extensive customization of agronomic management practices. A recently released enhanced version of SWAT, SWAT+, provides three main advantages over the historically used SWAT model for pesticide risks assessments of flowing water bodies: (1) the ability to directly simulate pesticide metabolite formation and environmental fate, (2) flexible spatial representation of landscape features and their interactions, and (3) advanced agricultural management practices, including rule-based probabilistic pesticide applications. This research was conducted to evaluate these new SWAT+ features and to assess its ability to predict observed pesticide and metabolite concentrations. The evaluation was conducted based upon a comparison of the results from six different model configurations with high-resolution monitoring data. The results showed that (1) SWAT+ is able to simulate the formation of degradate compounds and predict resulting concentrations in surface water, (2) an accurate representation of both surface and subsurface transport processes for pesticide exposure assessments is important, and (3) an appropriate degree of realism can be achieved with rule-based probabilistic pesticide application timing if information about the annual percent crop treated, a typical application rate, and a typical application window is available.

### 3.17.T-03 The Effect of Alternative Synthetic Hydrographs when Quantifying Pesticide Mitigation Efficiencies with VFSSMOD in Exposure Assessments

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The higher-tier regulatory pesticide risk assessments (EU FOCUS, US-EPA, Canada PMRA, etc.) use the model PRZM to calculate edge of the field surface flow, sediment and pesticide runoff mass in daily time steps within long-term simulations. VFSSMOD is currently available for the calculation of vegetative filter strip (VFS) pesticide mitigation within these regulatory frameworks. Critical to this analysis is that the inputs required to run VFSSMOD for each daily event simulation in long-term assessments are those already available within the regulatory risk analysis framework. For the case of rainfall data, in some instances, hourly rainfall records exist for regulatory agroecological scenarios, where in other cases only daily totals are available. Quantifying VFS efficiency requires consideration of the flow and transport dynamics within the filter during the storm. For this, VFSSMOD operates at the sub-hourly time step so PRZM daily field outputs used as VFSSMOD inputs must be distributed during the storm. Different alternatives could be considered but these can affect the realism and accuracy of the calculations and the results of the regulatory assessment. This study compares 3 approaches for distributing daily rainfall and field runoff based on standard storm durations for the specific regulatory scenario: two simplified (rectangular and triangular shapes) and a more physical approach used as benchmark for comparison. A previous VFSSMOD application case study is used to analyze the relative effect of the choice of hydrograph and rainfall distribution for a wide range of VFS sizes, 2 soil types dominant in the study area, storms magnitude (return periods) and duration, and pesticide characteristics (Koc). Statistical and global sensitivity analyses show that the triangular distribution is a good compromise in terms of simplicity of implementation, computational time and accuracy of calculated values for reductions of runoff, sediment and pesticide in the VFS. This supports its use in the regularly pesticide mitigation assessments with VFSSMOD.

### 3.17.T-04 Harmonised Framework for the Spatially Distributed Leaching Modelling of Pesticides Initiative: A 2023 update

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Spatially distributed leaching modelling (SDLM) of pesticides is a methodology to estimate leaching potential over a large spatial extent such as national or European. SDLM can help setting groundwater monitoring programs in context. It is described in the FOCUS groundwater report and foreseen to be used as higher tier leaching risk assessment as well as supporting monitoring studies. SDLM is already used as a higher tier assessment in the national authorization procedure in some EU countries and will probably become more important in future.

At the SETAC Europe 2020 online meeting, the initiative was officially formalised as a SETAC working group, consisting of a triad of members from regulatory agencies, academia, and the private sector. A steering committee manages the effort to develop a harmonized framework for spatially distributed leaching modelling across Europe. They published a problem definition document describing the aim and scope of the work. In 2021 subgroups for Geodata and Modelling were established each with a specific focus. As the SDLM teams continue to work, this presentation provides an update to interested parties.

The Geodata subgroup is evaluating datasets that can be used to generate a spatial modelling scheme and associated scenarios. Data reviewed fall in several established EU INSPIRE knowledge base metadata categories such as Agricultural, Meteorological, Land Cover, Hydrography, Soil, and many other categories. The Geodata group primarily focusses on pan-European datasets that cover the EU27 and the UK. Data has now been selected in each of these categories, and the presentation will highlight some important decisions, including the use of a new organic matter map.

The modelling subgroup is evaluating the models to be used in the SDLM context. Specific attention is paid to runoff processes. The subgroup showed that it is possible to describe runoff in a harmonised way using the runoff curve number approach. The modelling subgroup is further investigating if it is necessary to incorporate artificial tile drainage as well as preferential flow into the framework. However, in contrast to runoff processes, there is currently no agreed method available for the simulation of these two processes.

### **3.17.T-05 Contextualisation of Groundwater Monitoring Detections of Plant Protection Products: Observations from Site Elucidations**

*Andrew C Newcombe, Virginia Houck, Katherine Brutscher and Leah Martineau, Arcadis*

To support re-registration and environmental stewardship objectives, Arcadis has collected public monitoring data on more than 200 active substances and metabolites from many sources across Europe. More than 13 million analytical records have been identified by conducting database searches or contacting regulatory agencies and organisations that collect these data. For certain active substances and metabolites, elevated groundwater detections or exceedances of regulatory thresholds have been observed in the identified data. To better understand groundwater detections for select active substances, Arcadis has performed more than 140 detailed site elucidations across 12 European countries. Site elucidation activities included: 1) Determination of the sampling location type, quality and integrity, and protection measures. 2) Identification of nearby surface water and groundwater features, estimation of slope and direction, identification of any erosion or runoff channels, drainage features, and potential conduits to groundwater. 3) Documentation of karstic features, rock outcrops, and springs. 4) Interviews with farmers to collect historical product usage data on agricultural fields within the groundwater catchment area or 1,000-m radius of the sampling location. 5) Delineation of the treated field boundaries and locations of pesticide mixer/load, chemical storage, and farm machinery storage areas.

Data collected from each site elucidation was evaluated to determine if the potential cause of the groundwater detection or exceedance could be attributed to i) conventional leaching processes ii) point source contamination or iii) shortcut leaching pathways. From observations and data recorded during these site elucidations, a significant number of monitoring locations with groundwater detections or exceedances were classified as having a high possibility of a shortcut leaching pathway or potential point source contamination that contributed to the detections observed. Findings from the site elucidations demonstrate the need to assess the utility and quality of public monitoring data generated. Publicly available monitoring data for active substances and their metabolites provide important insight and knowledge about their leaching potential under actual use conditions and valuable information for use in assessing risk to groundwater. However, the quality of these monitoring data can vary greatly, which needs to be considered when used for regulatory risk assessment purposes.

### **3.17.P Measuring, Modelling, and Monitoring the Environmental Fate and Exposure of Pesticides**

#### **3.17.P-Tu257 Long-range atmospheric transport of currently-used pesticides over Europe**

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Currently-used pesticides (CUPs) are semi-volatile organic compounds that have been widely used in agriculture over the last 30 years at the global scale. Several CUPs were detected in air at remote locations such as the Arctic, showcasing their potential for long-range atmospheric transport. However, for most CUPs, their potential for long-range atmospheric transport has never been investigated. The aims of this study are (i) to report on the atmospheric levels of CUPs on the regional European scale and (ii) to identify the CUPs prone to long-range atmospheric transport.

To this end, a sampling campaign took place simultaneously at 29 sites in 17 European countries in spring 2020. The sampling sites were defined as rural (n=16), coastal (4), mountain (6) and polar (3) based on their geographical location and land use. About 120 samples were collected using a low or high-volume air sampler and were extracted and analysed by chromatographic methods coupled to mass spectrometry. In total, 76 CUPs were analysed in these samples including 35 herbicides, 22 insecticides and 19 fungicides.

Among the 76 CUPs targeted, 58 were quantified in the European atmosphere. In particular, six fungicides, six herbicides and one insecticide, which are all, except one, authorized for agricultural use in Europe, were found in more than 50% of the sampling sites. Overall, 22 out of the 76 CUPs investigated in this study were identified as prone to long-range atmospheric transport.

Indeed, at the polar sites, 19 CUPs were present in the air. In addition, in the five samples collected within the free troposphere of the mountain sites, 14 CUPs were quantified, of which 11 that were also found at the polar sites.

Out of the 22 CUPs identified as prone to long-range atmospheric transport, 15 are currently authorized for agricultural use in Europe, suggesting a need to revise the registration criteria in terms of long-range atmospheric transport.

### **3.17.P-Tu258 The Use of Chemical Plant Protection Products In Industrial Scale Vegetable Production in Finland: A Longitudinal Study**

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Pesticide usage data in the EU member states must be collected in accordance with the Community legislation concerning statistics on plant protection products (PPPs). However, the EU's public pesticide data still concerns only sales data. Our longitudinal study (17 years) utilizes a database that contains records of the use of PPPs on vegetable farms in southwestern Finland. The data was obtained from the local long-established food processing company which gave confidential rights to use the data to the Natural Resources Institute Finland (Luke). Here, we report, for the first time, the exact quantities of PPPs used in carrot, potato, swede, and fresh pea production in southwestern Finland from 2003 to 2019. Fresh peas and swede represent very low or decreasing use of PPPs, respectively. The number of fungicide treatments per field showed an increasing trend in potato, despite per unit area fungicide treatments having not increased. In carrots, insecticide and herbicide spray frequencies increased more than treatment volumes. The results of this study form a basis for analyzing ecotoxicological risks of PPP use in the studied crops because usage and spray frequencies alone do not convey the risk levels accurately.

### **3.17.P-Tu259 Spatial and Temporal Patterns of Pesticides Among Other Organic Pollutants in Seasonal Pools**

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Seasonal pools are defined as ephemeral wetlands that fill with water during the rainy season and dry out completely or mostly during the dry season. These water bodies support distinctive biodiversity and have great importance in conserving endemic species. Yet, due to anthropogenic stress, their occurrence has dramatically declined around the globe. In addition, surface water and runoff are increasingly contaminated with pollutants including microplastics, pesticides, pharmaceuticals etc. This may strongly impact the pools, which function as sinks for compounds washed with surface runoff and shallow underground flows. Although seasonal pools are gaining attention in the research literature, there is very limited information on the transport and dynamic of organic pollutants in them. This contrasts with their potential to serve as an efficient representative environmental model system for understanding pollutant mixtures transport mechanisms, dynamics and fate in aquatic environments. This study aims to shed light on the spatial and temporal dynamics of pollutant mixtures, mainly pesticides, and examine the parameters controlling these dynamics in seasonal pools located in Israel. Therefore, sediment, water and plant tissue samples were collected from 12 pools across Israel throughout one year. Preliminary results indicate that pollutant residues are present in the sediments and the water of all the pools, even those that seem separated from direct human influence. Plant protection products constituted the largest functional group of organic pollutants found in sediment and water during wintertime (47% and 38% respectively). A total of 25 different plant protection products were found in the sediments, ranging from zero in an isolated pool to 8-10 in pools adjacent to agricultural areas. On the other hand, 44 plant protection products were found in the winter sampling water, ranging between 6 to 30 different pollutants in each pool. The incompatibility in pools' ranking by plant protection products variety

between sediment and water requires further consideration. Additional data from the spring sampling will allow us to examine and discuss the temporal changes and the effect of other pools' characteristics such as vegetation presence. The presentation will discuss the spatial and temporal dynamics of pesticides with the aim to shed light on mechanisms that may provide authorities with science-based tools for managing runoff and seasonal pools.

### **3.17.P-Tu260 Water and Pesticide Transfers in Undisturbed Soil Columns Sampled From a Stagnic Luvisol and a Vermic Umbrisol Both Cultivated Under Conventional And Conservation Agriculture**

**Sixtine Cueff<sup>1</sup>**, Lionel Alletto<sup>2</sup>, Marjolaine Bourdat-Deschamps<sup>3</sup>, Pierre Benoit<sup>3</sup> and Valérie Pot<sup>3</sup>, (1)Plant Protection Products – Impact and Assessment, Agroscope, Switzerland, (2)University of Toulouse, France, (3)Université Paris-Saclay, INRAE, AgroParisTech, France

Conservation agriculture consists of a combination of (i) a strong reduction of tillage, (ii) the diversification of crop rotations, and (iii) a permanent soil cover by living plants or residues. Under such practices, mechanical disturbances are minimised and the water and organic pollutant dynamics are deeply modified. In particular, a “vertical dominant” functioning is favoured, thus limiting erosion and runoff (behind surface water contamination). The risk of groundwater contamination is, however, generally favoured and even more problematic when the increase in weed pressure, due to reduced tillage, can lead to a greater use of herbicides.

The objective of our study was to characterise water and pesticide transfers in two soils from the South West of France (Pyrénées-Atlantique and Gers regions) managed under contrasted agricultural practices (regularly tilled vs. conservation agriculture). To do so, we performed percolation experiment on undisturbed soil columns sampled from the surface horizon. A molluscicide (metolachlor) and two herbicides (nicosulfuron and mesotrione) frequently detected in the rivers of the studied fields catchment area were used. They were applied all together with a conservative water tracer. Columns were submitted to a series of two rainfall events (high and low intensities) separated by a two-days flow interruption, while maintained under unsaturated conditions (- 80 cm).

Preferential flows were evidenced at both sites and for both agricultural managements, but breakthrough curves showed a clearly different transport pattern between sites. At the Pyrénées-Atlantique site, behaviours were partly related to the pesticide sorption properties. Nicosulfuron and mesotrione were delayed compared to the tracer and slightly more under conservation agriculture. At the Gers site, we observed an early and simultaneous breakthrough of all substances under both management (independently of their sorption properties), suggesting a strong degree of preferential flow. At this site, experiments were carried out on shorter columns for the regularly tilled plot since a low conductive plow pan (evidenced by X-ray tomography) prevented flow beyond 20 cm. Due to this plow pan, surface and sub-surface runoff (observed in the field) are likely to be induced. Conservation agriculture practices performed for almost two decades seem, however, to have restored transfers to a 30 cm depth (at least), favouring the risk of groundwater contamination.

### **3.17.P-Tu261 Evidence for Aged Sorption to Be Used in Combination with Field Degradation Studies in Regulatory Assessments**

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In the EU Aged Sorption Guidance (SANTE/12586/2020) the use of field DegT50 values in combination with aged sorption parameters is only possible to a very limited extent. If the leaching assessment for a PPP is based on field DegT50 values only, it is not recommended by the current guidance to derive a DegT50<sub>equilibrium</sub> (rescaling of DegT50) for input in exposure models in combination with aged sorption parameters ( $f_{NE}$ ,  $k_{des}$ ) as described for laboratory DegT50 values. This virtually prevents the mitigating effect of aged sorption on leaching and limits the applicability of the aged sorption guidance in combination with field DegT50 to very few cases.

However, the guidance mentions that “Industry is preparing evidence for aged sorption in field studies and this option should replace the current recommendations as soon as appropriate guidance has been developed and tested”.

Therefore, additional field studies were evaluated to show the relevance of aged sorption in the field. The evaluation was mainly based on the comparison of observed concentration depth profiles versus FOCUS-PEARL simulations with and without consideration of aged sorption. Where possible, site-specific aged sorption parameters from laboratory studies with the respective field soils were used. The depth to which 95% of the residues are found was defined as metric for comparison. It was found that in all studies and sites the simulated soil profiles without aged sorption significantly overpredict the leaching of the substances. When considering aged sorption in the simulation the leaching was mostly still overpredicted but to a lesser extent which confirms the relevance of aged sorption in the field. In addition, the increase of apparent  $K_d$ -values from field and laboratory aged sorption studies with the same soils were compared with the result that the effects of aged sorption in field soils are similar to what is observed in laboratory studies.

### **3.17.P-Tu262 Temporal Trends of Legacy Chlorinated Pollutants in Various Fish Species From Norwegian Marine Areas**

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Fish stocks in Norwegian marine areas have been heavily impacted by various human activities in the recent decades and may be vulnerable to impacts of chemical contaminants even at relatively low levels. Several chlorinated persistent organic pollutants (POPs) as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCH), hexachlorobenzene (HCB), *trans*-nonachlor (TNC) have been included in the monitoring program of the Institute of Marine Research (IMR) carried out in Norwegian marine areas since the 1990-s. Sampling of various marine species was carried out with IMR's own research vessels during the summer-autumn period every 3rd year in the North Sea or the Barents Sea. Approximately

25 fish of each species were collected at a single or several locations in the same area and analysed at IMR using an accredited analytical method.

Liver of Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*) from the studied marine areas was found to have low to moderate levels of POPs, on average lower or slightly exceeding the established threshold values. The time trends have demonstrated a significant decrease with time for all the POPs (based on lipid weight) in the Barents Sea samples, where the levels in the 1990-s were considerably higher than in the later years. On the contrary, no significant decreasing trends were as a rule found in the North Sea for most POPs excluding HCH. The time series for the North Sea is from 2005 onwards, which may explain the absence of the decrease. It seems that the decrease has mainly occurred in 1990s and the levels have somewhat stabilized in the later years.

Sediment samples from the Barents Sea mostly show trace levels of the chlorinated POPs. The levels are correspondingly low in deepwater prawn (*Pandalus borealis*), capelin (*Mallotus villosus*), Atlantic herring (*Clupea harengus*), and are somewhat higher in polar cod (*Boreogadus saida*). There is however a significant decrease in POPs levels with time since the 1990-s also in these species. The levels are considerably higher in haddock and are the highest in Atlantic cod among all the studied species.

The observed time trends demonstrate a difficulty with removing these highly persistent pollutants from the environment once they have entered it. Biomagnification through different trophic levels is apparent and represents a danger for species occupying higher trophic levels.

### 3.17.P-Tu263 Occurrence and Distribution of Pesticides in Air at Two Agricultural Areas in Europe

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The ever-increasing problem of pesticide resistances and the inclusion of some pesticides in the Stockholm Convention due to their PBT (persistence, bioaccumulation and toxicity) properties and their potential to undergo long-range atmospheric transport has led to the application of newly discovered pesticides. These so-called currently-used pesticides (CUPs) can reach the atmosphere during their application due to spray drift or through post-application emissions such as volatilization. Some of these pesticides also occur in the air far from their application sites, even in such remote areas as the arctic. Information on the occurrence and distribution of these CUPs and their transport behavior are still limited.

This study was conducted within the framework of the EU project SPRINT. It investigates the occurrence and distribution of a wide range of pesticides in air at two agricultural areas in Europe (Portugal and the Netherlands) during a time period of one year (April 2021 until June 2022).

100 air samples were taken using high-volume air samplers. For the sampling of pesticides in the gaseous phase, PUF/XAD-2 cartridges were used, for the sampling of pesticides in the particle phase, glass-fibre filters (GFFs) were used.

The analytical method included 340 different pesticides, among them organochlorine pesticides, CUPs and 45 metabolites. PUF/XAD-2 cartridges were extracted by cold-column extraction with dichloromethane. GFFs were extracted using the QuEChERS approach. For GC analysis, a clean-up step using a dispersive SPE was performed. Instrumental analysis was performed by liquid chromatography coupled to a time-of-flight mass spectrometer and gas chromatography coupled to a tandem mass-spectrometer.

This study adds experimental data on the occurrence and fate of a wide range of pesticides in air. For pesticides found in both air phases, their distribution between the gaseous and particle phase can be determined by calculating gas-particle partitioning coefficients. Results show concentrations for several pesticides in the low pg/m<sup>3</sup> to ng/m<sup>3</sup> range. Highest concentrations of pesticides were found during the pesticide application period in spring and summer. Seasonal variations in pesticide concentrations and composition can be identified.

The results contribute to identifying pesticides to be considered in future work when investigating potential health impacts resulting from the exposure of humans to pesticides in the environment.

### 3.17.P-Tu264 Adaptive Neuro-Fuzzy Inference System Modelling of Pesticides and Heavy Metals Adsorption Coefficients in Soils

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Soil plays host to a multitude of chemicals, either applied on purpose or accidentally deposited from a variety of sources. Once on the soil surface, these contaminants are subject to vagaries of processes. Their persistence in soil, and migration to surface and groundwater, in particular, are problems of environmental concerns which require the knowledge of their sorption characteristics. Many mathematical models and/ or screening tools are described in the literature for evaluating the migration and leaching potentials of chemical contaminants in soils, using their distribution coefficients ( $K_d$  and  $K_{oc}$ ). These parameters are usually obtained from classical laboratory or field experiments. Not only is this approach rigorous and expensive, it could also be fraught with many errors. Thus, developing an accurate expert system for in-silico quantitation of these distribution coefficients is much desirable. Though the concept of linear solvation energy (i.e., polyparameter linear free energy relationships, pp-LFERS) presents accurate method, it suffers some limitations. Our literature search has shown that artificial intelligence (AI) techniques such as the adaptive neuro-fuzzy inference systems (ANFIS) have proven to be very accurate for prediction of partition coefficients of chemicals in near fields but have not been used for soils. Hence why we chose this method. We obtained experimental data from adsorption of five (5) substituted urea herbicides (linuron, diuron, monuron, isoproturon and chlorotoluron) and three toxic metals (Pb, Cd and Cu) in several differently composed natural topsoils. Based on the established correlations, nine variables were selected as input vectors with the adsorption quantity ( $Q_e$ ) as output vector. For each organic and inorganic systems as well as for

the integrated organic-metals systems, 255 ANFIS models were developed, using 10-fold cross-validation. These models were compared with similarly developed multiple linear regressions (MLRs), using three error metrics – mean average error (MAE), root mean square error (RMSE), and coefficient of determination ( $R^2$ ). ANFIS delivered models that satisfied our search not only for the most accurate prediction but also for the smallest combinations of input vectors to construct the models, with high accuracy and interpretability trade-off. These models have been proposed for in-silico predictions of distribution coefficients of a variety of environmentally relevant chemicals in soils.

### 3.17.P-Tu265 Technical Challenges In Soil Photolysis Studies Using SETAC 1995 And/Or Draft OECD Guideline

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Data on photolysis of chemicals in soil are required by Commission Regulation EU 283/2013 for the registration of plant protection products. The SETAC (1995) guideline is the regulatory relevant guideline, while the OECD draft guideline on soil photolysis (2002) has never been implemented. A key difference between the two guidelines is that the SETAC guideline uses air-dried soils, while the OECD draft guideline recommends air dried or moist soils (75% of the field capacity). Based on both guidelines, a thin layer (2mm) of soil is prepared in a quartz glass tray and applied with the test item. The applied soil is incubated in an irradiation unit with continuous irradiation of 75W/m<sup>2</sup> for a period of 10-15d. While performing this test, specific technical challenges have been identified that can influence the test validity. One of the issues is maintenance of the temperature of 20±2°C in the soil layer. Normally, a cooling unit (cryostat) is placed under the test vessel to cool the soil layer which is heated by the irradiation from top. This leads to a very high temperature gradient in the 2 mm soil layer (>> 10°C) and only the position and geometry of the temperature sensor decides on the temperature measured. Thus, the temperature recorded is no reliable validity criterium. Another concern is the drying of the moist soil under flow through conditions, which is a rapid and not reproducible process. This is due to the extreme high energy input by irradiation and cannot be avoided. The aim of this study is 1) to optimize the study setup used in the soil photolysis study and to investigate on a robust and reliable validity criterion instead of defining a temperature that cannot be measured properly. 2) to perform a literature review followed by lab experiments comparing the extent of photodegradation when dry or moist soil is used.

### 3.17.P-Tu266 Derivation of Active Substance Content in Heubach Dust from the Seed Dressing Rate for the Assessment of the Exposure to Abraded Dust when Sowing Treated Seeds

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The EFSA PPR Panel made already in 2004 explicit reference to the relevance of the exposure route “abraded dust drift deposition” in non-target areas when sowing treated seeds. However, the data available at that time were not sufficient to propose a calculation of the predicted exposure in the environment due to drift and deposition of abraded dust from seeds treated with plant protection products. After incidences with bees dying from abraded dust from treated maize seed in Germany 2008, exposure studies were conducted and guidance documents (GD) to assess and manage the risks were drafted (SANCO Working Group).

The current draft GD for Seed Treatment (Part Risk Assessment, version 17 from September 2021) includes a broad data basis on seed quality, especially for cereals. The exposure route by dust drift deposition on the ground and in vertical structures in non-target areas is dependent on crop, sowing density and seed quality can be expressed as amount of abraded dust (in the so-called “Heubach value”) and the active substance (a.s.) content in Heubach dust released from treated seeds (so called “Heubach a.s.”). To identify and characterize the possible relationship between the seed dressing rate and the content of a.s. in the dust, comparable data sets from cereal tests of 2011 and 2019 were combined and analyzed.

The target variable “% a.s. in dust” is binomially distributed in this dataset. A generalised linear model was used on logit transformed data. The data set showed a relationship between the concentration of a.s. in dust, the amount of abraded Heubach dust and the seed dressing rate. Previously defined reference values for professional seed treatment facilities regarding the concentration of the active substance is 10%. Following the regression model, seed dressing rates below 22 g a.s./180 kg seeds benefit from the refinement option. For these seed dressing rates, the estimation can be used to reference the quality control to dust abrasion, in cases when only a limited number of data on the content of a.s. in abraded dust are available.

So far, a sufficient data set exists for cereals. With the provision of similar data sets for other seeds types, more crop specific approaches and an extension to other crop seeds and granules are possible. This could facilitate the quality management in seed treatment while keeping the same level of protection for the environment.

### 3.17.P-Tu267 On the use of sScience-based Thresholds for the Interpretation of Environmental Monitoring Data: Demonstrating that Glyphosate Residues in European Water Pose No Risk to Ecosystems or Human Health

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Pesticide authorization in Europe requires an overview and assessment of environmental monitoring data, typically compiled from publicly accessible online data repositories and reports. Such information provides valuable insight into the state of the environment, emerging issues, and possible impacts. However, caution is required in the collation and interpretation of such data.

In order to determine whether remedial actions are necessary to ensure protection of human and environmental health, the underpinning basis for threshold limits should be considered in light of whether arbitrarily established, or science-based and data-derived. Environmental and human health endpoints to establish such levels for glyphosate (GLY) will be discussed and compared with current regulatory values in Europe and the United States.

A GLY Annex I renewal dossier was recently submitted in Europe. Public monitoring data collection/analysis for all Member States across environmental compartments included: 1) Surface Water (SW): comprehensive dataset (>300K samples from >15K sites) versus the regulatory acceptable concentration (RAC) of 400 µg/L, shows very high regulatory compliance (>99.99%). No official environmental quality standard (EQS) threshold has been set at the European level so far. 2) Groundwater: comprehensive dataset (>251K samples from >40K sites) versus the EU arbitrary regulatory threshold (0.1 µg/L) shows very high regulatory compliance rates (>99.4%). Excluding a small number of high outlier concentrations indicated maximum concentrations well below the SW RAC (for groundwater fed ecosystems) and the lifetime health-based Acceptable Daily Intake (LTHBADI) concentration (1500 µg/L). 3) Drinking Water: smaller dataset (>9.5K samples from >3.7K sites) versus the EU regulatory arbitrary threshold (0.1 µg/L) shows very high regulatory compliance (>99.9%) and is in good agreement with a large number of aggregated data summaries in published reports. Where threshold exceedances occur, the maximum concentrations are low and well below the LTHBADI concentration. This is unsurprising as GLY is readily removed by conventional water treatment processes already in place to ensure microbiological safety.

Analysis of a comprehensive database of GLY residue analyses within the public monitoring of national and regional environment agencies in Europe with robust science-based thresholds suggest no issues for the state of the environment, nor to human health *via* drinking water.

### 3.17.P-Tu268 Pesticides Screening on Water and Soils along Mekong River in Cambodia

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The massive use of pesticides has been causing great concern mainly regarding environmental contamination especially aquatic ecosystems including water, soil etc (1). Pesticides are detected in surface waters, close to agricultural sites, in concentrations from low ng l<sup>-1</sup> to high µ l<sup>-1</sup> (2). Pesticide usage in Cambodia is high, reflecting the importance of agriculture. There is no pesticide manufacturing in Cambodia and so the pesticide in Cambodia is entirely based on the importing from Vietnam, Thai, and China. All pesticides that are legally imported must follow the Cambodian legislation regarding quality assurance and labelling in Khmer language, a practice that results in lowering risk of misuse. However, it has been shown that illegally pesticides are used, which pose higher risk because the farmers don't understand the instruction written in the language of the origin of the pesticide. Nevertheless, the pesticides composition and prevalence, and the spread of pesticides in the environment have a few studied in Cambodia, severely limiting the possibility of targeted public health interventions.

This study aims to assess pesticide residue prevalence and composition in Cambodia. Water and soil samples along the Mekong River in Cambodia were analyzed for 65 pesticide residues by using liquid chromatography coupled with mass spectrometry methods. In total, 288 samples of water and soil (16 sampling sites within the four provinces along) were extracted by using solid-phase extraction and a modified Quick Easy Cheap Effective Rugged Safe protocol in combination with Ultrasonic-Assisted Extraction. The ecological risk assessment was also performed by calculating the risk quotient (RQ).

We plan to present the average concentration of detected pesticide residues in water and soil, and the value of risk quotient of detected pesticide residues in water and soil.

### 3.17.P-Tu269 Seasonal Monitoring of the Presence of Glyphosate and its Metabolite AMPA in Surface Water Bodies of the Puglia Region (Italy)

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Glyphosate is the active ingredient in several commercial products, and is currently the most widely used herbicide on the planet with an estimated worldwide use of 750.000 tons. Since 2015, it has been classified as a "probably carcinogenic to humans" by IARC. As for other pesticides, also for glyphosate and its main metabolite AMPA, the Puglia Region carries out the monitoring of surface water bodies in the implementation of Directive 2000/60/EC and D.Lgs. n.152/2006. These standards include the obligation to monitor and classify water according to environmental quality objectives. Monitoring of surface water bodies is linked to the six-year duration of the Management Plans and Water Protection Plans. This study presents the results of the Glyphosate and AMPA analytical activities provided by the seasonal monitoring program for the years 2019-2021.

Approximately 98 surface water samples of Puglia Region were analysed for each campaign. The physicochemical properties of the herbicide and the consequent analytical difficulties led to the development of a better retention and separation method. A pre-column derivatization with FMOC-Cl was applied and coupled to UHPLC-MS/MS. In 2019, Glyphosate and AMPA were present seasonally in 44 sampling stations with a maximum value of 4 µg/L for Glyphosate detected at the Cervaro river and 38 µg/L for AMPA found at the Canal of Contessa site. Both compounds were recorded in the spring season. Average annual values were 0.2 µg/L for Glyphosate and 1.2 µg/L for AMPA. In 2020, Glyphosate and AMPA were investigated in 98 stations with a maximum value of 17.85 µg/L for Glyphosate in the Triolo stream and 105 µg/L for AMPA in Cervaro river. Both were recorded in the summer season. Average annual values were 0.2 µg/L for Glyphosate and 1.3 µg/L for AMPA. In 2021, Glyphosate and its metabolite were analysed in 103 stations with a maximum value of 0.9 µg/L for Glyphosate in the Asso stream and 11 µg/L for AMPA in the Contessa canal. Both were recorded in the spring season. Average annual values were 0.1 µg/L for Glyphosate and 0.7 µg/L for AMPA. Overall, the results show a widespread presence of the herbicide and its metabolite throughout the region with minors differences among seasons over the years. This surely reflects a consistent use of the pesticide. More in-depth studies will be undertaken to identify the most significant sources in order to implement proper management of environmental resources.



### 3.17.P-Tu270 Sourcing and Interpretation of Water Monitoring Data for Crop Protection Substances, Biocides, and Pharmaceuticals in Europe

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For the registration of active substances for use in Plant Protection Products within the European Union, the current data requirements (EC 283/2013) state that;

*“Available monitoring data concerning fate and behaviour of the active substance and relevant metabolites, breakdown and reaction products in soil, groundwater, surface water, sediment and air shall be reported.”*

However, little to no guidance has been provided on how to support this request. In recent years, a number of member states have begun to request these data as part of the renewal of approval process for active substances under the crop protection legislation. While, overall databases exist that provide data for a number of crop protection, biocides and pharmaceutical substances (for both European and non-European countries), the amount of data available in these databases has been found to be lacking for a number of substances.

In this poster we will compare the availability of national water monitoring data across the EU (including the United Kingdom and Norway) and comment on the quality and usefulness of the available data.

We will discuss how we have compiled this information into useable format and how best to visualise the information.

### 3.17.P-Tu271 Occurrence of Plant Protection Products in Spanish surface water

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A wide variety of plant protection products (PPPs) have been used in agriculture and landscape maintenance for controlling weeds (herbicides), insects (insecticides) or fungi (fungicides) and other pests, and for reducing competition from weeds since the middle of the 20<sup>th</sup> century.

Water pollution due to the use of PPPs in agriculture is a priority environmental issue and a cause of major global concern. The main objective of the European Water Framework Directive 2000/60/EC is to protect the water quality in order to prevent environmental and human health damage. Some PPPs present Environmental Quality Standards (AA-EQS) values and others have been included in the surface water watch list in 2022. Thus, this study evaluates the current trends of PPPs in river water to reflect the ecological status in an area of high representativeness such as the Tagus River watershed in its Spanish section.

A total of 25 river water samples were collected during 2020 and 2021 at 13 sampling sites in the Tagus river basin to determine the presence of 172 PPPs. The sampling locations were selected to represent different typologies of the Tagus River watershed and the target compounds (herbicides, fungicides, insecticides and some of their degradation products) included a wide range of physicochemical characteristics, uses and toxicity.

PPPs were detected in all water samples with a large dispersion in concentrations, from 0.2 ng/L to 47 ng/L (median). A total of 109 PPPs were found in the surface water. The range of compounds detected in a single sample was from 2 to 90 (median: 70), corresponding the highest value to a sampling point located in an urban and industrial area. PPPs with higher incidence (detection frequency > 90%) were carbendazim (20.2 ng/L, median), fluopyram (1.3), imidacloprid (47.1), 2,4-D (20.9), acetamiprid (12.3), azoxystrobin (7.5), azoxystrobin-O-desmethyl (37.9), diuron (27.6), metolachlor (6.7), tebuconazole (5.4) and terbutylazine-desethyl (4.3).

The reported concentrations are of interest since these surface waters may be treated by water treatment plants to generate tap water, where the presence of PPPs could have human health implications.

### 3.17.P-Tu272 Pesticides in Aquatic Ecosystems in the Andean Region of South America: Occurrence and Ecological Risk Assessment in the Uco Valley (Mendoza, Argentina).

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Chemical pollution constitutes one of the major drivers of biodiversity loss in freshwater ecosystems. It has been widely demonstrated that pesticides can reach aquatic ecosystems and affect the species that inhabit them. In Argentina, the major agriculture production is related to extensive crops (e.g. soybean, corn, wheat) and the pesticides monitoring efforts are mostly focused on water bodies related to agroecosystems located in the plains of Pampas region. However, there are other regional crops with economic relevance that include different management strategies and occur in ecosystems with differential climatic and hydrogeological characteristics. This is the case of the Uco Valley (Mendoza province, Argentina) in the Andean mountains, with highly technified production of vine, fruits and vegetables, but with scarce information on its environmental effects on water bodies. In this context, this study aimed on quantify pesticides in aquatic ecosystems of the Uco Valley and on develop an ecological risk assessment (ERA). For this purpose, two sampling campaigns (spring/ summer) in five sites with different land uses were carried out. Surface water samples were obtained (by triplicate) and current use pesticides were extracted with SPE columns and quantified with UPLC-MS/MS. A total of 72 analytes (pesticides and metabolites) were studied, and risk quotients (RQs) were calculated for each site/season. Several herbicides, fungicides and insecticides were detected in the different sites, with different patterns likely related to land use and hydrological conditions of water courses. A total of 16 analytes were detected, being atrazine and atrazine-deisopropyl, carbendazim, DMA, imidacloprid, tebuconazole, terbutylazine-2-hydroxy and terbutryn the most ubiquitous. The RQs calculation showed that the pesticides reported in the Uco Valley could implicate

medium/ high risk on organisms inhabiting in its aquatic ecosystems. Particularly, the herbicide terbutryn and the fungicide carbendazim were the major contributors to risk and, in some cases, also the fungicides tebuconazole and difenoconazole and the insecticide diazinon. These results constitute, for the author's knowledge, the first report of ERA for pesticides present in aquatic ecosystems of Uco Valley. The ecological risk assessment would be complemented with higher tier analysis, including different methodological approaches that include the evaluation of *in situ* effects on biological communities.

### **3.17.P-Tu273 Aquatic Risk Assessment at Catchment Scale – Case Study on Herbicide Exposure From Spray Drift and Drainage in Arable Crops in Belgium**

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Landscape scale modelling is considered to be important for future environmental risk assessment of plant protection products (PPP). Through assessing the risk at landscape levels and accounting for spatial and/or temporal variability, the realism of environmental risk assessment can be improved. A previous study quantified spatial and temporal risks for a strongly sorbing insecticide, for which entry via spray drift could be assumed as the only entry route. Yet, for mobile compounds, like most herbicides, drainage and runoff are potentially relevant entry routes to nearby surface water. The present study will evaluate spatially and temporally distributed surface water concentrations, due to spray drift deposition and drainage entries from application of a herbicide in arable crops within a meso scale river basin in Belgium. To this end a PEARL component will be added to the (previously developed) landscape model to calculate the emission via the soil and the drains to surface water (runoff is incorporated at later stage).

This landscape model integrates hydrology, PPP use, PPP drift deposition, pesticide fate, and effect components. A component incorporating the PEARL model is added to calculate pesticide drainage leaching to the nearby reach for each field in which PPP is applied. In the landscape model the water flows and depths calculated by the hydrological programming library 'Catchment Modelling Framework (CMF)' are used by modules for spray drift (xDrift) e-fate (Cascade\_TOXSWA) and drainage (PEARL). The fate of the pesticide is simulated with an hourly timestep for all reaches in the catchment.

Spatial and temporal surface water concentrations are calculated on the catchment scale. By including the drainage entry route in the landscape model the fate of mobile compounds and estimation of effects can be done more realistically. The emission from drain pipes is associated with lower concentrations than spray drift deposition, but the total mass emitted is much considered to be higher. The impact of chronic exposure to aquatic organisms of these substances may therefore be of relevance. The modifications make the modular framework for landscape risk assessment, suitable for a broader range of pesticides and allows for simulation of effects to aquatic macro-invertebrates, as well as e.g., macrophytes.

### **3.17.P-Tu274 Glyphosate Concentrations in Groundwater in Southern Spain – Causes and Stewardship Measures**

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Glyphosate (GLY) public groundwater monitoring data from EU member states show high compliance rate (> 99 %) with the regulatory groundwater concentration threshold of 0.1 µg/L. For Spain this compliance rate is lower (93.6 %). Available data from the heterogeneous monitoring programs of the River Basins show exceedances in the region of Andalusia, southern Spain. To address the situation locally, the Glyphosate Renewal Group (GRG) initiated an investigation to assess the reason for this lower compliance rate.

As part of the study, 45 monitoring sites that showed the most exceedances, were subject to an elucidation. The results for Andalusia showed that most monitoring wells are located in rural areas, dominated by agriculture and that many of the wells are not adequate to assess the leaching behaviour of plant protection products (PPPs). Most of the monitoring sites assessed were not dedicated groundwater monitoring wells and used by farmers for irrigation, abstraction and in parts for mixing and loading of PPPs. Apart from the wells' deficiencies the elucidation identified likely reasons for the elevated GLY concentrations in groundwater in Andalusia. For most sites specific farming practices are likely cause for the GLY detections in groundwater. Also, for few uses additional label indications will be an improvement to avoid potential contributions as sources of GLY in groundwater.

Farmer surveys in Andalusia were performed to obtain information on GLY usage and application practices considering different agronomic settings (crop, farm sizes and management practices). Although in Spain all farmers before using crop protection products have received compulsory official training, the GRG findings show that there is opportunity for improvement in training skills related to handling, application, and documentation of plant production products. Particular on smaller farms it is relevant to train how to calculate and document the correct application rate; increase awareness about the different water contamination sources and improve the product label interpretation.

The elucidation and farmer surveys indicate that responsible stewardship program and farmer involvement can have a significant positive effect and thus increase environmental compliance at local level. Therefore, the GRG, Spanish industry association and local authority are committed to continue supporting, developing advocacy activities and training on water protection and safe use of PPPs.

### **3.17.P-Tu275 PERSAM Tier 3a with PELMO: What Happens to the Metabolite PECs if Parent Defaults are not Adapted?**

**Mareike Albrecht**, Sarah Becker, Nils Lange, Inga Schreiter and Steffen Schubert, RIFCON, Germany

Following the tiered approach described in the new EFSA Soil Guidance (2017), PECsoil values need to be calculated using the software tool PERSAM (at Tier 1 and 2) and the models FOCUS PELMO and FOCUS PEARL at Tier 3a of the risk assessment. At Tier 3a, the output generated by PERSAM is transferred to the higher tier models PELMO and PEARL via a transfer file. Thereby, additional substance specific parameters, e.g. the Freundlich exponent and plant uptake factor, must be specified by the user. In case of PELMO, this can be done via its graphical user interface (GUI) before the psm-file is generated. However, for the metabolite's run, the GUI does not provide the option to put in the necessary parameters for the parent substance but only for the respective metabolite. Instead, these parameters for the parent are set to default and need to be adapted in each individual psm-file, which is a laborious and error-prone task.

Interestingly, the guideline does not specify that the parameters of a parent substance need to be adapted when running a metabolite, which leads to the question whether this manual step is necessary at all, and, if yes, to which extent the parent parameters affect the resulting PECsoil values of the metabolite.

Thus, we studied a selection of pesticides using EFSA agreed endpoints and compared the output between manually changed parameters and defaults. From this comparison we can learn whether or not this time-consuming manual adaption of psm-files is scientifically needed or if this step can just be skipped in future risk assessments.

### 3.17.P-Tu276 Differences between the EFSA OM Map and the SoilGrids OM Map for Arable Land Europe and Potential Implications for Soil Risk Assessment

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In 2017, the European Food Safety Authority (EFSA) published a new guidance with four tiers for the exposure assessment of plant protection products (PPPs) in soil (EFSA, 2017) together with the PERSAM (Persistence in Soil Analytical Model) tool. To be able to calculate the 95<sup>th</sup> spatial percentile of the estimated soil concentration distribution in PERSAM, spatial data for crop growing, climate parameters and soil properties in Europe are considered. For the organic matter content in soil the EFSA (2012) OM map is used which is based on the organic carbon content information available in the ESDB (European Soil Data Base). The OC content of the ESDB map was converted to organic matter using a fixed constant (OC content x 1.724 = OM content) assuming that soil organic matter contains 58% carbon. In a final step a pedotransfer function (Tiktak et al. 2002) was used to estimate the bulk density (BD) of the soil exclusively based on the OM content. That means finally that OM and BD are based on estimations and not on measurements. It is already stated in the soil guidance document (EFSA 2017) that a true alternative for that OM data set was not available at this point in time. However, it was also stated, that once a better alternative is available, this new data set should be used to update PERSAM. Better alternative spatial data in the EU are becoming available (for example the SoilGrids OM map published in 2022 by De Sousa et al. 2022). In a GIS (GeoInformation System) analysis, the BD content given in EFSA (2012) and SoilGrids (BD calculated based on the same pedotransfer function as for EFSA) is compared with the measured BD data (LUCAS 2018) from 9000 sampling points across Europe. The comparison was done for arable land only. While overall estimated BD from EFSA and measured BD from LUCAS (2018) were mostly comparable, except for some outliers, regionally some differences were present. In comparison, calculated BD from SoilGrids (2018) different more from measured values from LUCAS (2018). As for the comparison between BD from EFSA (2012) and LUCAS (2018) there were notable regional differences. In general, the estimated BD data from EFSA seemed to better reflect measured BD values from LUCAS (2018). By updating the PERSAM software with more accurate OM and BD data (e.g. mixture of EFSA and SoilGrids based on the region), the new underlying spatial soil data would correspond more to the real soil conditions in Europe.

### 3.17.P-Tu277 Spatially Distributed Numerical Soil Exposure Modelling – First Experiences and Concepts

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The soil exposure assessment has been recently revised by EFSA in a new guidance document (EFSA Soil GD). An entirely new tiered assessment scheme considering new modelling software, spatial environmental datasets and process-based physical modelling are proposed. In a previous impact assessment by Croplife Europe, predicted environmental concentrations in soil (PECsoil) were calculated using the current approach by FOCUS and the new one proposed by EFSA to evaluate 56 active ingredients and metabolites covering a broad range of substance properties and uses. The failure rate of the lower tier risk assessment for soil organism increased from 10% (current FOCUS approach) to 67%, 58% and 36% at Tier1, Tier-2 and Tier-3A (EFSA) respectively. The higher failure rates originate from high PECsoil values triggered, among other conservative factors, by atypical pedoclimatic scenarios, representative of agronomically unrealistic soil conditions (e.g. organic carbon content).

The EFSA Soil GD defines a higher tier refinement using spatially distributed numerical models (Tier-3B) allowing for a more realistic spatial assessment and relevant exposure scenarios selection. However, it further states that agreed software tools and methods are required to apply Tier-3B. Thus, the objective of this work is to present first experiences to establish Tier-3B. Firstly, spatial input datasets are evaluated comparing the well-accepted but partly outdated datasets used by the EFSA Soil GD with up-to-date information on environmental conditions and land use based on remote sensing and observations. Secondly, the scenario selection procedure for the calculation of relevant PECsoil is investigated with respect to the refined modelling approach and the more realistic assessment. Thirdly, the technical applicability of Tier-3B is evaluated from the perspective of (i) the requirements for the underlying technical infrastructure to execute the numerical models FOCUS PEARL and PELMO at EU scale (from multi-core local machines to cloud computing) and (ii) the software requirements to process European scale spatiotemporal data by using specific tools to store (e.g. hierarchical data format - HDF) and process (e.g. Python NumPy and Dask) large scale multidimensional data (often referred as 'datacubes' or 'hypercubes').

### 3.17.P-Tu278 A Study on the Impact of Surfactants on the Transport of Plant Protection Products (PPP) Under Different Hydrodynamic Conditions

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In this study, we investigated multicomponent reactive solute transport in top agricultural soils. The impact of the concentration of surfactants/adjuvants on the transport of plant protection products (PPP) under different hydrodynamic conditions was studied numerically. These investigations aimed to contribute to better mechanistic understanding for determination of the application rate of PPPs and bioavailability of PPPs in the soil, which ultimately are used for ensuring efficiency in production in support of sustainable agriculture.

Numerical simulations of competitive sorption with linear, Langmuir, and Freundlich models were performed using the PHREEQC package. Adsorption, absorption, and desorption mechanisms were included in the modeling system to capture all interactions between solute-solute and soil-solute simultaneously. Factors that potentially can influence the sorption of PPPs in soil including soil texture, the active ingredient, and adjuvant properties have been examined. Furthermore, the effect of hydrodynamics including boundary conditions on sorption behavior have been studied.

The numerical model was extended, based on the attachment-detachment concept, to simulate colloidal-facilitated chemical transport. The preliminary numerical results indicate that the presence of the colloid particles in the system may influence the transport of adjuvant and active ingredients in soil depending on the boundary conditions and soil properties.

### 3.17.P-Tu279 Application of Spatially Distributed Leaching Modelling to Quantify FOCUS Tier 3b Reduction Factors for the National Situation in UK, FR, and NL

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The European FOCUS groundwater models and scenarios are intended to assess the potential for movement of pesticide active substances and their metabolites to groundwater considering a Tiered approach. At Tier 1, the default FOCUS GW scenarios are employed together with mean values for the different model input parameter (e.g., geometric mean DT50 and Koc; arithmetic mean 1/n). At Tier 2a parameter refinements can be made so that an observed pesticide-specific behavior can be incorporated in the assessment (e.g., consideration of pH dependent adsorption and degradation). At Tier 3b spatially distributed leaching modelling approaches can be employed to reflect the spatial and temporal variability of the underlying processes. However, a harmonized approach for such assessments at EU MS level does not currently exist.

A FOCUS Tier 3b modelling framework was employed to evaluate the leaching behavior at the national level in the UK, FR and NL considering: 1) annual foliar fungicide application in cereals (BBCH 30-39, 80% crop interception) 2) parent with Tier 1 input; metabolite with Tier 2a input for pH dependent behavior; 3) FOCUS groundwater model PEARL 3.3.3 and PEARL 4.4.4 as employed by the individual spatial model frameworks; 4) exposure evaluation based on the overall 90<sup>th</sup> risk percentile 5) computation of SDLM reduction factors based on comparison to the worst-case FOCUS scenario (Hamburg).

As no “ready to use” Tier 3b approach or tool currently exists, the individual approaches had to be chosen with the methodology being in detail somewhat country-specific. As a first step, the SDLM approach used the specific data already available at the national level of the three countries. Soil information for England and Wales is taken from the SEISMIC 2.0 database, corresponding data for Scotland is taken from the Scottish Soils Knowledge and Information Base. Soil information for France is taken from the FROGS 2.2.2.2 and FROGS 3.3.3.3 model systems whereas corresponding information for the Netherlands is taken from the Dutch GeoPEARL 333 and GeoPEARL 444 model versions.

Outcome of the Tier3b modelling and resulting Tier3b refinement factors compared to lower Tiers are presented and discussed. The outcome clearly demonstrates a significant safety margin of the lower Tiers.

### 3.17.P-Tu280 Assessment of the Protectiveness of Predicted Environmental Concentrations in Surface Water Based on a Vulnerable, High-Use-Intensity Catchment in Belgium

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In the process of pesticide approval in the European Union it has to be shown by the applicant that an unacceptable risk for the aquatic environment by the active substance can be excluded. For this aim, standardised models, scenarios and agreed pesticide input parameters must be used to calculate the exposure as required by the FORUM for the Co-ordination of pesticide fate models and their Use (FOCUS). The use of so-called realistic worst-case scenarios in combination with average compound input parameters ensures that the predicted exposure is at least protective of 90 percent of all spatio-temporal exposure situations. In this study, the question should be answered whether these Predicted Environmental Concentrations in Surface Water (PEC<sub>sw</sub>) are actually protective in the sense of their definition by comparison to Measured Environmental Concentrations in surface water (MEC<sub>sw</sub>) from a vulnerable, high-use intensity catchment under “real world” conditions in Europe.

The present monitoring dataset contains surface water concentrations of 12 herbicides and one metabolite on a daily basis along with application rates, application dates and the treated crops over 3.5 years for a catchment in Belgium at two different sampling points. One sampling point represents the whole surface water outlet of the catchment, while the second one only drains the eastern portion of the catchment area.

The behaviour of the compounds in the regulatory standard watershed was simulated by a combination of the models MACRO or PRZM with the aquatic fate model TOXSWA to simulate PEC<sub>sw</sub> due to drainage, runoff and spray drift entries. The regulatory

PEC<sub>sw</sub> reflects an “edge-of-field” situation. There, stream scenarios (for Belgium D4 and R1) receive direct drainage or runoff fluxes from a treated 1 ha field adjacent to the watercourse and in addition baseflow from a 100-ha upstream catchment, where 20% of the area is treated at the same time. Since the sampling points in the monitoring study are downstream or even at the outlet of the catchment without any known application in direct vicinity, the simulated concentration only in the upstream catchment without an edge-of-field contribution, i.e. the PEC<sub>upstream</sub>, was considered as a potentially better metric, in addition to the regulatory edge-of-field PEC<sub>sw</sub> for a comparison with the MEC<sub>sw</sub>.

### 3.17.P-Tu281 Drainage Mitigation by Soil Litter Layer under Conservation Tillage

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Continuous conservation tillage is often associated with an increase of pesticide leaching and transport to drains, due to increased water infiltration and preferential flow processes. However, the scientific literature shows different effects of conservation tillage practices on drainage and leaching of pesticides indicating great complexity and interplay of relevant environmental processes. Conservation tillage and direct drilling operations also result in a build-up of previous crop residues on top of the soil as litter or mulch. This litter acts as an extra layer which initially intercepts applied pesticides and where degradation may be faster and adsorption more effective than on bare soil, leaving less substance to be transported deeper into the soil. This may not only counterbalance the effect of increased transport due to preferential flow but may even lead to a substantial decrease of drainage and leaching concentrations and could be thus used as an effective mitigation measure.

The aim of this work was to assess the effect of a soil litter layer on the surface water predicted environmental concentrations using standard regulatory modelling tools. First results show that depending on tested scenario and application period the decrease of predicted environmental concentrations in drainage scenarios may range between 30% and 99% compared to the standard FOCUS calculation. These findings suggest the need to further investigate leaf litter layer processes under conservation tillage and their potential effect on pesticides concentrations in the environment. A realistic field study has been started which may bring more in-depth process understanding and provide further proof in favor of the tested hypothesis.

### 3.17.P-Tu282 Comparison of Regulatory Waterbody Models

*Amy Ritter and Mark Cheplick, Waterborne Environmental*

This presentation will show a comparison of predicted environmental concentrations (PECs) using regulatory waterbody models used in the United States, EU, and Andean countries. A single application to EU winter cereal and potato crop scenarios were run with winPRZM (Pesticide Root Zone Model) simulating the runoff/erosion from a field transferring into a waterbody. Three different adsorption coefficients (Kocs) were modeled with half-lives of 100 days. The mass loads from winPRZM were input into three regulatory waterbody models: TOXic substances in Surface Waters (TOXSWA), Variable Volume Water Model (VVWM), and EXposure Analysis Modeling System (EXAMS). The comparison shows the range of variation or similarity between PECs for pond and stream environments between the different models. While an attempt was made to create identical parameterization of inputs for the models there are some algorithmic differences for the stream environment that cannot be duplicated in all three models. These differences lead to more variation in the PECs when simulating higher Kocs and the stream environment. The pond environment did not exhibit this trend and results were much more aligned.

### 3.17.P-Tu283 Comparison of Measured and Predicted Environmental Concentrations of Chemicals Using The Surface Water Model STEPS

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Predicted environmental concentrations (PEC) result from mathematical fate models and are used as an indicator for the occurrence of pesticides and other chemicals in the environment. There is a plenty of fate models, differing amongst others in the level of detail of their process descriptions. The FOCUS TOXSWA model as the standard model used in the EU for pesticide registration has a high level of complexity in process descriptions, specifically concerning the sediment compartment. In contrast, models exist with less detailed process descriptions and thus less input data. An example for this is the STEPS 1-2 model developed by Michael Klein from the German Fraunhofer IME, which is kept much simpler than TOXSWA and based on a single equation only. To investigate the impact of the level of detail of process descriptions on the quality of the PEC, we used the STEPS 1-2 model to generate PEC values. STEPS 1-2 implements different levels of complexity according to the FOCUS Tier 1 and Tier 2 risk assessment. First, Step 1 calculates a single loading to the water body, and in that sense “worst case” surface water and sediment concentrations without considering loss processes. Second, in Step 2 a series of individual loadings resulting from drift events is considered including basic loss processes following first-order kinetics. The predicted STEPS-derived Step 1 and Step 2 PEC estimations are then compared to measured environmental concentrations (MEC) as reported in a study about a comparison between PEC and MEC values based on the FOCUS TOXSWA model (Knaebel et al., 2012). Furthermore, PECs from STEPS are compared to FOCUS-TOXSWA-derived PEC values as reported in Knaebel et al. (2012) with the aim to explore the relation between the level of detail of the process description and the accuracy of model predictions of environmentally occurring pesticide concentrations. aim for understanding the basic relation between process details and accuracy of predictions, and to identify the relevant processes and parameters

### 3.17.P-Tu284 Primary Distribution Model of Pesticide for Japanese Paddy Field

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Life Cycle Assessment (LCA) studies on the toxicity assessment of pesticides are underway. The database used for the

calculation: Ecoinvent, has a scenario in which all the emissions are deposited in soil within agricultural land. Therefore, a model has been developed that divides the emission rate into compartments for air, freshwater, and soil. The latest model is a new version of the previous model with a different calculation method for each parcel. This study aims to understand the differences and issues in the calculation methods for each partition in the primary distribution between the conventional model and the latest model, and to develop a more appropriate method.

The models studied are the Japanese paddy field model based on the conventional model and the latest PestLCI consensus model. The setup of each primary distribution plot was investigated, and the estimation method for each plot was studied. Then I compared the emission factors calculated by both models using a common drift curve.

The Japanese paddy field model divides the emission plots into three categories: air, surface water, and leaves, while the PestLCI consensus model divides the emission plots into four categories: air, agriculture soil, crop, and off-field soil.

The estimation methods are the same for surface water and agriculture soil, leaves, and crops, but air and off-field soil are different. Specifically, the paddy field model assumes drift in the air and the PestLCI consensus model assumes deposition to off-field soils. It also estimates emission air rates based on the combination of application methods and target crops.

As a result, the off-field deposition fraction of the PestLCI consensus model is basically larger than the atmospheric fraction of the paddy field model. However, Aerial differs in the PestLCI consensus model, about 20% is deposited, while in the paddy field, 4% to 40% is dispersed to the atmosphere.

This is because the variables in each model are different. For the paddy field mode are the application width, the size of the farmland. However, the PestLCI consensus model variable is the ratio of farm to off-farm width, integrated over the drift curve coverage.

Based on the above results, I will improve airborne and off-field deposition and propose a more appropriate model.

### **3.17.P-Tu285 TOXSWA Simulates Pesticide Exposure in Intermittently Dry Ponds Hosting Amphibians**

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Amphibians often breed in small water bodies in or adjacent to agricultural fields. Field applications of pesticides may pose harm to the amphibians, because pesticides may enter the water bodies via spray drift deposition, surface runoff or drainage from the treated fields. In the EU aquatic risk assessment, pesticide exposure is characterized by surface water scenarios that do not include the typical, small-sized amphibian breeding ponds. Current EU pond scenarios are not suitable as the simulated ponds are too large to represent realistic worst-case dimensions of amphibian ponds. Moreover they do not reflect the often highly variable or decreasing water depths in such ponds.

In 2020 the EU COST Action “PEsticide RiSk AssessMent for Amphibians and Reptiles” (PERIAMAR) (CA18221) was started to identify and fill knowledge gaps. One of the aims is to design ad-hoc scenarios to predict pesticide exposure concentrations in amphibian breeding ponds across the EU.

TOXSWA has been adapted to be able to simulate variable water depths in small ponds with sloping sides, using precipitation, evaporation, downward seepage and a minimum, 1-cm water depth to mimic “falling dry”. A pond scenario has been developed. Dimensions of the pond are based on requirements of artificially constructed amphibian breeding ponds in The Netherlands.

FOCUS R1 26-year weather data have been used for daily water temperature, rainfall and evaporation. The water depth of the pond was calibrated to fall dry once in five years. Example simulations for spray drift entries of a weakly sorbing and strongly sorbing pesticide show the significant impact of low water levels on aqueous concentrations in amphibian breeding ponds. A next step is to simulate runoff entries of the PRZM model.

### **3.17.P-Tu286 Spatial and Temporal Distribution of Herbicides along Railroad Tracks in Germany**

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Rail traffic places high demands on the functionality and safety of rail constructions, which are directly linked to systematic vegetation management. For decades, chemical vegetation control by means of herbicides, especially applied to the track bench and bed, has been one of the main control measures used in Germany. In earlier years, vegetation-free track systems were mostly achieved by using poorly degradable and environmentally hazardous herbicides. However, the properties of the herbicides used, such as adsorbability and biodegradability, as well as structural characteristics of the track substructure, led to pollutant accumulation in the different layers of the track body and in the environment. The objective of the present study is to identify dispersion patterns and fate of herbicides in and along track systems for a comprehensive environmental risk assessment. In contrast to other emitted pollutants, herbicide input into the track area occurs only once a year, which allows the degradation and dispersion behavior to be systematically investigated. For this purpose, soil samples were taken and analyzed at selected measuring points in a period of 0 to 90 days before and after vegetation control at different depths (0–10 cm and 20–30 cm) as well as different distances (2.5 m and 5.0 m) from the track center line. In addition, precipitation-dependent seepage water samples as well as surface and groundwater samples were collected and characterized. The analyses focused on chemical-analytical sample characterization, degradation behavior, and derivation of hazard potential using standardized test procedures and evaluation standards (ISO 14001). The results show that out of the formerly used herbicides, only a few active agents are still detectable in the soil. Due to their adsorption on the railway ballast and cohesive soils, a sufficient retention capacity is guaranteed and a possible hazard is limited to soils with a low depth to water table. For the currently approved herbicides (e.g., glyphosate), the highest risk potential was determined up to 10 days after herbicide application for runoff rainwater and surface water. Systematic spatial and temporal sampling of various environmental compartments provided an in-depth understanding of

contaminant dispersal occurring in and along railroad tracks. The investigations form the basis for an adapted environmental risk assessment of railway tracks and the optimization of hazard prediction models.

### **3.17.P-Tu287 Effect of stone content on alternative railway pesticide leaching in a coarse textured soil**

**Sixtine Cueff**, Roy Kasteel, Thomas Poiger and Ignaz Buerge, *Plant Protection Products – Impact and Assessment, Agroscope, Switzerland*

In Switzerland, only glyphosate is authorized to control weed pressure on railway tracks. However, due to its suspected carcinogenicity and upcoming ban in Europe, the Swiss Federal Railways is searching for alternatives with the objective to withdraw from usage by 2025. In the framework of a governmental project, many non-chemical alternatives are under study, as well as chemical ones relying on more eco-compatible substances with a minimum leaching to groundwater. For the latter part, Agroscope conducts a lysimeter experiment, evaluating the leaching behaviour of several alternatives to glyphosate. Lysimeters are filled with railway tracks substrates, collected from three different Swiss locations. These substrates are characterised by a high gravimetric stone content (32–69 %), which may deeply affect the soil hydraulic properties and thus the transport of solutes. However, to date, little is known about the reactive solutes transfer, such as pesticides, in these soils. The complex responses observed under natural climatic conditions in the lysimeters could complicate our understanding of the effect of stones on pesticide transfers. A complementary experimental-modelling study under controlled laboratory conditions (constant irrigation rate 2 cm/d, temperature 20°C and unsaturated conditions –15 cm) was then designed. We aim at evaluating the mobility of a water tracer (bromide ion) and six herbicides and some of their metabolites in repacked soil columns where we gradually increase the stone content (0, 20, 40, 60%). The herbicides were chosen to cover a range of leaching potential properties. All substances travelled rather rapidly (sometimes equally fast) through the soil columns under a ‘no stones’ condition. Considering the risk of an identical behaviour even at the lowest stone content (limiting our ability to understand the effect of stones on pesticide transfers), lower mobility substances were added for the following experiments with stones. Indeed, either due to the decrease of the soil transport volume or the presence of preferential flow pathways at the soil-stones interface, pesticide transfer is expected to be faster under “stones” conditions. Simultaneously, the presence of stones may create immobile areas that modify the physical and/or chemical non-equilibrium of herbicides, which has not been evidenced yet. When fitting the experimental breakthrough curves, a two-region model will then likely be the most suitable.

### **3.17.P-Tu288 Deposition of pesticides via spray drift in off-crop vegetation: A study of the vertical and horizontal distribution in flower strips**

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The authorization process for plant protection products (PPP) includes an assessment of adverse effects on non-target arthropods (NTA) both in the treated field and off the field (“off-crop” areas). While the exposure assessment for example aquatic species is well established, a solid experimental basis is still lacking for the exposure assessment in off-crop vegetation. Particularly, information about the distribution of PPP deposited in off-crop vegetation via spray drift is limited. One of the objectives of the Swiss Biodiversity Strategy and the Swiss Action Plan for Risk Reduction and Sustainable Use of Plant Protection Products (AP PPP) is therefore to further develop the risk assessment of PPP towards NTA in off-crop vegetation. To characterise the horizontal and vertical distribution of PPP deposited in off-crop vegetation via spray drift, we conducted extensive field experiments using a fluorescent tracer. The tracer was applied with a conventional boom sprayer onto a surrogate crop (artificial meadow). The spray drift in the adjacent vegetation (a 6 m wide flower strip) was collected on filter paper targets (area per target, 500 cm<sup>2</sup>) positioned at different heights (at the level of the leaf canopy, half way up and a few centimetres above the ground) and at distances of 1, 2, 3, and 6 m, respectively, from the edge of the treated field. Wind speed and direction were recorded with high temporal resolution during each tracer application. Data analysis was carried out using a linear mixed-effect model using R Studio. First results indicate similar dependence of spray drift on the distance from the edge of the field, both for spray drift deposition on ground level and on off-crop vegetation. In addition, interactions between different variables such as the pressure / nozzle type and wind speed are evaluated.

### **3.17.P-Tu289 Evaluation of Mechanistic Model Simulation of Field Study Spray Drift from Unmanned Aerial System**

**Michael F Winchell**<sup>1</sup>, Sebastian Castro-Tanzi<sup>1</sup>, Jane Tang<sup>2</sup>, Dan Martin<sup>3</sup>, Brad Fritz<sup>3</sup>, Yaning Yang<sup>2</sup> and **Hendrik Rathjens**<sup>4</sup>, (1)Stone Environmental, (2)Bayer AG - Crop Science Division, (3)USDA ARS, (4)Stone Environmental

Recently, with the increased use and availability of unmanned aerial systems (UAS) for application of agrochemical sprays, a better understanding of spray drift from these platforms has emerged as a particular area of interest from many stakeholders. The approaches to better understanding off-target spray drift from UAS are being addressed from both field study and modeling perspectives. Field studies are allowing direct measurement of spray drift deposition from UAS under a range of environmental and application conditions, and this data is necessary to validate mechanistic models under development for the simulation of these emerging types of applications. Two independent field studies were conducted to evaluate spray drift for varied droplet sizes and application conditions using two different UASs. These field datasets were then used to evaluate the predictions of spray drift deposition from a recently developed mechanistic model to simulate within spray block and off field drift and deposition from UAS agrochemical sprays. The sensitivity of the model simulation results to several input assumptions, including the parameterization of the UAS structural configuration and associated flow field, were assessed. The field study measurements and model simulation results were also compared with regulatory modeling results for traditional ground spray application platforms. The results of these comparisons will be presented along with field study results which demonstrate that best management



practices (BMPs), such as swath offset, are important in achieving targeted sprays and reducing off-target spray drift from UAS. Regulatory drift evaluation should also consider such BMPs to reflect field practices.

### **3.17.P-Tu290 Deriving triggering, persistence and modelling endpoints for GB risk assessment: a tool to simplify**

**Lily Elizabeth Summerton, Chloe Blackham and Emily Cumming, Chemicals Regulation Division, Health and Safety Executive, United Kingdom**

The derivation of DT50 values for use in the pesticide active substance risk assessment is a complex process. The available guidance encompasses several documents (for example, FOCUS (2014) kinetics guidance, DG SANCO Working Document (2012), EFSA (2014) DegT<sub>50</sub> guidance, FOCUSgw (2000; 2014) guidance) and therefore, the complete information required to select endpoints for trigger endpoints, modelling endpoints and persistent, bioaccumulative and toxic chemicals (PBT) assessments is not presented in its entirety in any one place. The process is further complicated by the wide range of factors that must be considered when selecting the endpoints (for example, biphasic behaviour, normalisation, pH dependence and statistical differences in laboratory and field DT50s). Accurate derivation of these endpoints for active substances and their associated metabolites is critical for conducting the PBT assessment, determining whether field studies are required, and calculating predicted environmental concentrations (PECs) used in the GB risk assessment for human health (PEC<sub>gw</sub>) and non-target organisms (PEC<sub>soil</sub> and PEC<sub>sw</sub>).

HSE has utilised both current and new guidance to produce a comprehensive flow map that standardises the selection of endpoints to determine if field studies are required, assess PBT criteria, and select input parameters for the respective PEC calculators and models. This visual tool breaks down the HSE approach into simple steps and is intended for the use of both applicants and evaluators.

The purpose of this poster is to provide clarification of the guidance for deriving trigger, PBT and modelling endpoints for GB. More specifically, it includes how to derive DT50 values from biphasic kinetic models, clarifies when values should be normalised, how to determine pH dependence and explains the use of the endpoint selector tool for laboratory vs. field DT50 values. Important considerations in the handling of the endpoints for parent and metabolites are also detailed. The poster presents an overall picture of the endpoint selection approach for GB risk assessment.

### **3.17.P-Tu291 Normalisation of laboratory and field degradation endpoints – modelled vs. measured data**

**Lily Elizabeth Summerton, Chloe Blackham and Emily Cumming, Chemicals Regulation Division, Health and Safety Executive, United Kingdom**

Temperature and moisture have a significant effect on the soil degradation rates of pesticides. Therefore, in cases where degradation studies are performed under non-standard conditions, it may be necessary to normalise the dissipation half-life (DT50) values to reference temperature and moisture conditions (20 ± 2°C and pF2). For example, DT50 values derived from laboratory studies should be normalised to reference conditions before being used in the environmental risk assessment, PBT assessment or used to determine if field studies are required. DT50 values derived from field studies should also be normalised when their intended use is for calculating PEC<sub>gw</sub> and PEC<sub>sw</sub> values. The Organisation for Economic Co-operation and Development method 307 states that laboratory studies should be carried out at 20 ± 2°C and soils adjusted to a pF of between 2 and 2.5. For normalisation to pF2 conditions, when measured pF2 values are not available, default inputs specified in FOCUS Ground Water (2014) guidance are used. These values, however, are approximations based on the generic properties of various soil types, and the guidance states that there is potential to produce anomalous results.

In addition, field residue data undergo time-step normalisation to derive modelling endpoints, as recommended in the FOCUS (2014) kinetics guidance. This process is required to determine a degradation rate that would be observed if temperature and moisture had been constant over the study duration. However, issues can arise as in-situ field monitoring is often unreliable (particularly soil moisture monitoring). Historically where significant gaps in the monitoring data occur (for example equipment malfunctions), soil temperature and moisture have been simulated using programmes such as PEARL and PERSIST.

This poster presents a HSE investigation into the significance of using default pF2 values and modelled temperature and moisture data when normalising laboratory and field data. Based on the results of this investigation, HSE provides recommendations on modelling soil moisture and temperature as well as minimising the impacts of using modelled data and default pF2 values in the normalisation of both laboratory and field DT50 values.

### **3.17.P-Tu292 Assessing the relevance of non-European field studies in GB pesticide active substance risk assessment**

**Chloe Blackham, Lily Elizabeth Summerton and Emily Cumming, Chemicals Regulation Division, Health and Safety Executive, United Kingdom**

Organisation for Economic Co-operation and Development (OECD; ENV/JM/MONO) (2016)<sup>6</sup> suggests that provided the conditions in a field dissipation study conducted in one country are representative of conditions in another country, the results of that study could be used in regulatory assessments in that other country. Therefore, field dissipation studies conducted at non-European sites can be accepted for use in European pesticide active substance risk assessments if the conditions during the study are representative of European conditions. To support this concept the OECD ENASGIPS tool was developed to consider North American and European Union (EU) conditions and uses an “ecoregion” approach to compare soil and climatic conditions. The appropriateness of this approach for the GB pesticide regulatory system is considered.



The ENASGIPS ecoregion similarity scoring system can be useful in demonstrating broad similarities between areas. However, the use of mean annual temperature and precipitation data within ENASGIPS, as well as mean soil pH and soil organic carbon, provides a non-specific comparison between broad geographical areas. In practice, the actual conditions at each study site should be comparable to European conditions. ASGIPS does not provide this detailed comparison and therefore is not used as the sole determinant as to whether conditions in a non-European field dissipation study can be considered comparable to GB conditions and thus if the study can be used for GB risk assessments.

HSE's Chemical Regulation Division has been applying the limited recommendations provided in the EFSA DegT50 guidance (2014) on assessing the relevance of non-EU field studies to GB risk assessments. The GB expectation is that information on the comparability of the site-specific soil and weather conditions during the actual course of the study in relation to European conditions is submitted as part of the active substance data package.

This poster aims to outline GB regulatory expectations. This poster describes an approach where at least monthly average air temperature and precipitation at each site is compared and the representativeness of the soil to European conditions is considered.

### **3.17.P-Tu293 C2D2: An Open-Source, Pan-European, Harmonised Crop Development Database for Use in Pesticide Exposure Modelling and Risk Assessment**

**Gregory Hughes**<sup>1,2</sup>, **Andrew Eatherall**<sup>3</sup>, **Michael Bird**<sup>4</sup>, **Jonathan Blake**<sup>5</sup>, **Philip R Branford**<sup>6</sup>, **Sebastian Gebler**<sup>7</sup>, **Anthony Lozano**<sup>8</sup>, **Paul Massey**<sup>9</sup>, **Gerald Reinken**<sup>10</sup> and **Liz Whitworth**<sup>5</sup>, (1)Cambridge Environmental Assessments (CEA), United Kingdom, (2)GeoSpatial Analytics, Wolverhampton, United Kingdom, (3)Corteva Agriscience, United Kingdom, (4)Syngenta, United Kingdom, (5)RSK ADAS, United Kingdom, (6)ADAMA Deutschland, Germany, (7)BASF SE, Germany, (8)Sumitomo Chemical Agro Europe SAS, France, (9)FMC Agricultural Solutions, Afghanistan, (10)Bayer AG - Crop Science Division, Germany

Determining appropriate pesticide application windows is an important part of pesticide exposure modelling. Increasingly, regulators are constraining application windows using the AppDate software. Given exposure assessments are a function of the application dates this software generates, it is important to understand how representative they are; however, an appropriate pan-European crop phenology dataset was previously unavailable for such an assessment. Efficacy trial datasets sourced from seven members have been harmonised to create the Crop Life Europe Crop Development Database (C2D2) that meets this need. The C2D2 spans two decades (2000–2020), >250 crops and 30 European countries.

Evaluation of C2D2 against an independent dataset (PEP725) showed good agreement for equivalent time periods, crop growth stages and geographical regions. This supports the underlying premise that efficacy trials are typically conducted in line with expected agronomic practice and are appropriate for this purpose. In addition, the data's alignment suggests that the data cleaning/processing has not affected the utility of the resulting dataset. C2D2 critically covers a fuller description of crop growth stages across a broader geographical region and is specifically aligned with the risk assessment procedures under 1107/2009/EC having standardised crop and FOCUSsw/gw scenario attribution.

Despite the large dataset compiled and the geographical coverage of C2D2, not all FOCUSsw/gw scenarios have sufficient data to facilitate comparison, with smaller scenarios, like FOCUSgw Porto, being under-represented. For those scenarios with sufficient data, significant differences between the CLE and AppDate crop development dates are often indicated over some/many growth stages suggesting that amendment of the existing representation of crop development within the risk assessment process may be required. While the provenance of the data underpinning AppDate crop development is not documented in the software manual and associated reports, much of the difference is ascribed to C2D2 comprising mostly post-2010 data describing crop development of modern crop cultivars bred to match current agronomic and weather constraints more closely.

The C2D2 will be made freely available under a Creative Commons licence to facilitate innovation in exposure science to allow for more accurate and realistic risk assessment leading to enhanced crop and environmental protection.

### **3.17.P-Tu294 A New European Dataset of Artificially Drained Agricultural Areas**

**Gregory Hughes**<sup>1</sup> and **Steven Anthony**<sup>2</sup>, (1)GeoSpatial Analytics, United Kingdom, (2)RSK ADAS, United Kingdom

The use of artificial pipe drainage in agriculture is an important control for soil waterlogging and shallow groundwater tables. A well-designed drainage system will reduce the risk of detrimental waterlogging to acceptable levels, increasing crop yields and the number of available machine working days. While the drains rapidly remove excess soil water they also facilitate the rapid movement of soil pollutants, like nutrients and pesticides, to surface water.

An inventory of the extent to which soils requiring drainage have actually been drained is an important input for spatially distributed models seeking to investigate the fate and behaviour of agricultural chemicals. In the absence of such data a gross assumption of complete drainage of all soils requiring drainage is made. This poster presents an updated digital map of the extent of pipe drainage in arable agriculture across Europe. The map is compiled from national sources of pipe drainage compiled using diverse approaches ranging from agricultural surveys of farmers/drainage contractors to digitised field maps of historic installations. For most countries the revised map represents a marked improvement over existing spatial datasets of artificial drainage. The poster also provides a summary of the age of drains installed in various countries as an indicator of the likelihood that drains are still operating optimally as is typically represented in chemical fate models.

### **3.17.P-Tu295 Analysis of Glyphosate, Glufosinate and AMPA in environmental water with direct injection**

**Benjamin Wuyts**<sup>1</sup>, **Janitha De Alwis**<sup>2</sup> and **Claudia Rathmann**<sup>3</sup>, (1)Waters Corporation, Belgium, (2)Food and environment, Waters

Corporation, United Kingdom, (3)Waters Corporation, Germany

Monitoring highly polar, small organic pesticides in waters as well as in food presents an analytical challenge. The purpose of this work was to demonstrate a direct injection ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the determination of highly polar anionic pesticides in drinking and environmental waters. The method performance study was completed on an ACQUITY UPLC I-Class PLUS™ System with a Xevo TQ Absolute™ mass spectrometry.

A method validation study was carried out on drinking, reservoir, river, and ground water. The method performance was assessed for glyphosate, AMPA, and glufosinate using 2 spike levels at 20 and 60 ng/L for all analytes, with 11 replicates through inter-day and intra-day testing. The coefficients of determination ( $r^2 > 0.999$ ) and residuals ( $< 10\%$ ) were all excellent. Average method performance for trueness was between 95 and 103% across all matrices, while the RSDs were below 11%.

This poster demonstrates the performance of a method, combining stable retention on Waters' Anionic Polar Pesticide Column (APPC), ultra-performance chromatography on the ACQUITY™ I-Class and absolute MS power with the Xevo™ TQ Absolute to provide a fast and robust solution to one of the most challenging and time-consuming applications in environmental analysis.

### 3.17.P-Tu296 DIRECT AQUEOUS ANALYSIS OF PESTICIDES AND PPCPS IN DRINKING AND BOTTLED WATER AT PARTS PER TRILLION LEVELS

**Daniel McMillan<sup>1</sup>**, Jianru Stahl-Zeng<sup>2</sup>, Bertram Nieland<sup>3</sup>, Jack Steed<sup>1</sup>, Ian Moore<sup>4</sup> and Philip Taylor<sup>1</sup>, (1)SCIEX, United Kingdom, (2)SCIEX, Germany, (3)SCIEX, Netherlands, (4)SCIEX, Canada

Drinking water analysis is subjected to extremely low and rigid prescribed consent values (PCV) for determinands. Water suppliers and utility companies need to ensure that the final water product they send out for bottling or into supply networks is safe and complies with state and country regulations. Water can be a challenging matrix, in particular if it is sourced from a river, lake or reservoir before entering the water treatment process.

A series of experiments was conducted in collaboration with Vitens Water Company, to test several types of water samples spiked with a mixed standard of 431 determinants, including pesticides, pharmaceuticals and personal care products.

Direct aqueous injection was performed of spiked water samples - MilliQ water, tap water, Evian bottled water and Fiji bottled water. The samples were spiked with a custom mix standard, containing 431 analytes. A series of 11 standards were made in the respective matrices at sequential concentrations from 0.1 to 500 ng/L. A collection of unknown blank samples was also included in this study. The analysis was performed using a SCIEX 7500 system, operated in both positive and negative electrospray ionization with fast polarity switching.

The analytical method presented shows ultra-high levels of sensitivity with LOD values of 0.1 ng/L reported. All data was acquired through direct aqueous injection with no SPE needed. Good chromatographic peak-to-peak separation was achieved with a total run time of 25 minutes. Even at low level concentrations, excellent levels of precision and accuracy were achieved (e.g. %CV  $\leq 6.76$  at 0.1 ng/L). There is scope to expand the number of analytes tested and apply this system to other individual workflows which demand very low limits of quantification. This method provides solutions for drinking water analysis and problematic determinands such as dicamba.

### 3.17.P-Tu297 Automated Workflow for At-Instrument Extraction and Analysis of Organochlorine Pesticides

**Bryan White** and Callum Morgan, Agilent Technologies, Inc., United Kingdom

Analysis of Organochlorine Pesticides (OCP's) in water is traditionally performed by solvent: solvent extraction and requires at least 100 ml of sample and a minimum of 25 ml of organic extraction solvent. A fully automated instrument solution for the analysis of OCPs in water has been developed and fully validated. The method requires only 15 ml of sample (with the addition of a small quantity salt) to be added to a 20 ml headspace vial. The rest of the sample preparation is fully automated utilizing a CTC Pal3 RTC autosampler, thus removing any human error from the process and requiring only 2 ml or organic solvent. Analysis is done utilizing concurrent mid column backflush on an Agilent 8890 GC coupled to a 7010 Triple Quad equipped with High Efficiency Source operating in EI mode. Linear calibrations ( $R^2 > 0.998$ ) for all analytes are achieved for calibrations from 0.01 to 0.25 ug/L. Excellent sensitivity is shown with all analytes having S: N  $> 50$  at the 0.01ug/L level. The poster details aspects of the automation and the tools used together with techniques to maximize robustness within the analysis.

### 3.17.P-Tu298 HIGH SENSITIVITY QUANTIFICATION OF CHLOROTHALONIL METABOLITES IN SURFACE, GROUND AND BOTTLED DRINKING WATER

**Michael Scherer<sup>1</sup>**, Jack Steed<sup>2</sup>, Aidan Harrison<sup>2</sup> and Jianru Stahl-Zeng<sup>3</sup>, (1)SCIEX, Switzerland, (2)SCIEX, United Kingdom, (3)SCIEX, Germany

Chlorothalonil is a fungicide that was widely used around the world for grain and vegetable cultivation. In Switzerland it was one of the top 10 agrochemicals used during the last few years. Due to increasing concerns about the toxicity of chlorothalonil and the discovery of several metabolites in the environment it was banned in 2020. The two main metabolites R471811 and R417888 were detected at high concentrations in ground and surface water. Therefore, there is a growing need to analyze and monitor these metabolites, to protect drinking water supplies, map the distribution, and to understand pathways and long-term behavior.

Different types of water such as ground water, surface water and drinking water should be analyzed to assess potential exposure. A total of 10 chlorothalonil metabolites were analyzed: R471811, R417888, SYN507900, R611968, chlorothalonil 4-hydroxy, SYN548580, SYN548581, R611965, R611553, and M7. Isotopically labelled internal standards (IS) were used for R471811 and R417888. A calibration curve from 0.1 to 1000 ng/L was prepared in mineral water. HPLC separation was performed on a Kinetex Polar C18 column (100 × 4.6 mm, 2.6  $\mu$ m). Thflow rate was 0.9 mL/min. The injection volume was 20  $\mu$ L and the total LC runtime was 17 minutes.

The analytical method presented shows quantification of 10 chlorothalonil metabolites down to 0.1 ng/L. Samples were quantified using an external standard calibration in bottled drinking water, with specificity confirmed using ion ratios and retention time confirmation. Recoveries were between 90 to 105% for all water matrices analyzed and excellent reproducibility was observed at 10 ng/L with RSD values between 0.4% and 4.3%. R471811 and R417888 were observed frequently, and sometimes at very high concentrations. 5 other metabolites were detected at lower concentrations, and 3 metabolites were not present in any sample investigated here. Accurate results for early eluting polar compounds such as chlorothalonil R471811 can be obtained in several water types with low matrix and salt content such as drinking water and lake water without isotopically labelled internal standards. The method described here allows the monitoring and analysis of these metabolites, to protect drinking water supplies, to map the distribution, and to understand pathways and long-term behavior.

### 3.17.P-Tu299 LC-MS/MS analysis of triazole derivative metabolites in complex matrices

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Every year, 2.6 mln metric tons of pesticides contaminate the environment. Most of them, (85%), are used in agriculture. There is no doubt that pesticides play an essential role in maintaining a high level of food production, but on the other hand, they can get to the agricultural crops, and their presence in food should be constantly monitored. Triazoles as systemic fungicides are used for control and treatment of a wide range of fungal diseases on a wide variety of crops. All pesticides belonging to the triazoles group contain the 1,2,4-triazole moiety and are metabolized to four main common metabolites known as triazole derivative metabolites (TDMs): 1,2,4-triazole (TRZ), triazole alanine (TA), triazole acetic acid (TAA), and triazole lactic acid (TLA), which commonly occur in plant materials. Hence, it is extremely important to develop analytical methods which allow the determination of triazole derivative metabolites in the plant material. Due to their physical-chemical properties, TDMs analysis in the complex matrices is very difficult. The main aim of this study was to develop a method for the analysis of these analytes that is quick, easy to handle, and enables quantification at a residue level of 0.01 mg/kg.

The analytical methods were developed and fully validated for the determination of triazole derivative metabolites. The developed methods offer the determination of triazole derivative metabolite, combining selectivity, high resolution capacity and fast analysis time with the advantages of simple, rapid and reliable extraction procedures. Validation criteria (linearity, the regression residual (di), selectivity and specificity, precision, matrix effect, accuracy, and limit of quantification and detection) were performed in compliance with the SANTE/2020/12830, Rev. 1 guideline, ensuring the suitability of the method.

### 3.17.P-Tu300 Development of an environmental monitoring database “Biocides in the environment”

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They are part of our daily lives: biocidal products - whether as disinfectants, insecticides, or rodenticides, but also as preservatives for wood or textiles. Their widespread use is closely linked to unavoidable emissions into the environment and leads to potential risks to non-target organisms in various environmental compartments.

The question of how serious the problem of biocide contamination of the environment really is and whether risk assessment and risk management actually lead to the prevention of unacceptable concentrations can be examined by reviewing environmental monitoring data. However, a European collection of biocide monitoring data in various environmental compartments and the evaluation of these data does not yet exist.

Therefore, our objective is to develop the database “Biocides in the environment”: It contains environmental monitoring data for biocidal active substances and relevant metabolites acquired between 2015 and 2022 from all over Europe. The data were collected by an intensive literature research in scientific publications and reports, but also measured environmental concentrations from different monitoring databases (e.g. NORMAN) were included. As a stand-alone module in the database “Information System on Chemicals” (ChemInfo) of the German Federal Government and the German Federal States, the data will be open to the general public and decision-makers in politics and society and will raise awareness of possible environmental hazards of biocides. Through the evaluation of the data, priority substances can be identified as well as substances with missing monitoring data which is useful for regulatory authorities and scientific communities. A geographic mapping of the data allows to identify regions with high densities of measured data for biocides and regions with unsatisfying data. Also, information on the degree of coverage of the single compartments is gained. Emission pathways and fate of the active substances used in different product types may be reproduced by means of analysing the monitoring data. Concentration trends over time mirror the changing use of the biocidal products throughout the examined time span. Furthermore, the database can be a useful tool for post-authorisation monitoring to evaluate the environmental exposure and risk assessment and to review or adapt risk reduction measures in the context of the regulation of biocidal active substances.

### 3.17.P-Tu301 Preliminary Report of the Exposure to Glyphosate and Glufosinate of a Male Population in the Province of Córdoba (Argentina)

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Argentina is one of the world leaders in the production and export of agricultural products. The volume of pesticides application increased during the last decades, being the herbicides, mainly glyphosate (GLY), the top sold products in the phytosanitary market. In recent years a notable increase in sells of herbicides no-GLY, as glufosinate (GLU), has been reported due to the weed resistance. In the province of Córdoba, the production of wheat, corn and soybeans requires the use of GLY and GLU. However, physicochemical characteristics of GLY, its main metabolite, aminomethylphosphonic acid (AMPA), and GLU make their detection particularly complex at low concentration levels, and the exposure of the local population remains unknown.

The aim of this work was to develop a sensitive technique to detect and quantify GLY, AMPA, and GLU in plasma and urine of a male population with different scenarios of exposure residing in the province of Córdoba.

Urine and plasma samples were adequately pre-treated, derivatized with FMOC-Cl and further extracted by solid phase extraction. Identification and quantification of the herbicides were carried out using high performance liquid chromatography (HPLC) system coupled to a triple-quadrupole mass spectrometer equipped with a heated electrospray ionization source (TSQ-Quantiva). Then, urine and plasma samples from 31 subjects were analysed; 12 of them were people from the general population, and 19 were occupationally exposed to pesticides.

The developed analytical procedures reached limits of quantification between 0.3 and 5.1 ng/mL with recoveries between 39 and 84 %. The most frequently detected analyte in plasma was GLY, followed by GLU (32 and 10 % in the total population, respectively). In urine, the most frequently detected herbicide was GLU, followed by GLY (13 and 6 % of the total population, respectively). The metabolite, AMPA, was the less frequently detected in both matrices. No differences between groups of subjects were found regarding the median concentration of the analytes.

In conclusion, the analytical methodologies for the quantification of GLY, AMPA, and GLU were adequate to analyze the biological samples. This study is the first report of concentration of GLU in plasma and urine in human population.

### **3.17.PC Measuring, Modelling, and Monitoring the Environmental Fate and Exposure of Pesticides**

#### **3.17.P-Tu259 Spatial and Temporal Patterns of Pesticides Among Other Organic Pollutants in Seasonal Pools**

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Seasonal pools are defined as ephemeral wetlands that fill with water during the rainy season and dry out completely or mostly during the dry season. These water bodies support distinctive biodiversity and have great importance in conserving endemic species. Yet, due to anthropogenic stress, their occurrence has dramatically declined around the globe. In addition, surface water and runoff are increasingly contaminated with pollutants including microplastics, pesticides, and pharmaceuticals etc. This may strongly impact the pools, which function as sinks for compounds washed with surface runoff and shallow underground flows. Although seasonal pools are gaining attention in the research literature, there is very limited information on the transport and dynamic of organic pollutants in them. This contrasts with their potential to serve as an efficient representative environmental model system for understanding pollutant mixtures transport mechanisms, dynamics and fate in aquatic environments. This study aims to shed light on the spatial and temporal dynamics of pollutant mixtures, (mainly pesticides), and examine the parameters controlling these dynamics in seasonal pools located in Israel. Therefore, sediment, water and plant tissue samples were collected from 12 pools across Israel throughout one year. Preliminary results indicate that pollutant residues are present in the sediments and the water of all the pools, even those that seem separated from direct human influence. Plant protection products constituted the largest functional group of organic pollutants found in sediment and water during wintertime (47% and 38% respectively). A total of 25 different plant protection products were found in the sediments, ranging from zero in an isolated pool to 8 to 10 in pools adjacent to agricultural areas. On the other hand, 44 plant protection products were found in the winter sampling water, ranging between 6 to 30 different pollutants in each pool. The incompatibility in pools' ranking by plant protection products variety between sediment and water requires further consideration. Additional data from the spring sampling will allow us to examine and discuss the temporal changes and the effect of other pools' characteristics such as vegetation presence. The presentation will discuss the spatial and temporal dynamics of pesticides with the aim to shed light on mechanisms that may provide authorities with science-based tools for managing runoff and seasonal pools.

#### **3.17.P-Tu260 Water and pesticide transfers in undisturbed soil columns sampled from a Stagnic Luvisol and a Vermic Umbrisol both cultivated under conventional and conservation agriculture**

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Conservation agriculture consists of a combination of (i) a strong reduction of tillage, (ii) the diversification of crop rotations, and (iii) a permanent soil cover by living plants or residues. Under such practices, mechanical disturbances are minimised and the water and organic pollutant dynamics are deeply modified. In particular, a “vertical dominant” functioning is favoured, thus limiting erosion and runoff (behind surface water contamination). The risk of groundwater contamination is, however, generally favoured and even more problematic when the increase in weed pressure, due to reduced tillage, can lead to a greater use of herbicides.

The objective of our study was to characterise water and pesticide transfers in two soils from the South West of France (Pyrénées-Atlantique and Gers regions) managed under contrasted agricultural practices (regularly tilled vs. conservation agriculture). To do so, we performed a percolation experiment on undisturbed soil columns sampled from the surface horizon. A molluscicide (metaldehyde) and two herbicides (nicosulfuron and mesotrione) frequently detected in the rivers of the studied fields catchment

area were used. They were applied all together with a conservative water tracer. Columns were subjected to a series of two rainfall events (high and low intensities) separated by a two-day flow interruption, while maintained under unsaturated conditions (–80 cm).

Preferential flows were shown at both sites and for both agricultural managements, but breakthrough curves showed a clearly different transport pattern between sites. At the Pyrénées-Atlantique site, behaviours were partly related to the pesticide sorption properties. Nicosulfuron and mesotrione were delayed compared to the tracer and slightly more under conservation agriculture. At the Gers site, we observed an early and simultaneous breakthrough of all substances under both management (independently of their sorption properties), suggesting a strong degree of preferential flow. At this site, experiments were carried out on shorter columns for the regularly tilled plot since a low conductive plow pan (evidenced by X-ray tomography) prevented flow beyond 20 cm. Due to this plow plan, surface and sub-surface runoff (observed in the field) are likely to be induced. Conservation agriculture practices performed for almost two decades seem, however, to have restored transfers to a 30 cm depth (at least), favouring the risk of groundwater contamination.

### **3.17.P-Tu261 Evidence for aged sorption to be used in combination with field degradation studies in regulatory assessments**

*Ian Hardy, Battelle UK, United Kingdom*

In the EU Aged Sorption Guidance (SANTE/12586/2020) the use of field DegT50 values in combination with aged sorption parameters is only possible to an extremely limited extent. If the leaching assessment for a PPP is based on field DegT50 values only, it is not recommended by the current guidance to derive a DegT50<sub>equilibrium</sub> (rescaling of DegT50) for input in exposure models in combination with aged sorption parameters ( $f_{NE}$ ,  $k_{des}$ ) as described for laboratory DegT50 values. This virtually prevents the mitigating effect of aged sorption on leaching and limits the applicability of the aged sorption guidance in combination with field DegT50 to very few cases.

However, the guidance mentions that “Industry is preparing evidence for aged sorption in field studies and this option should replace the current recommendations as soon as appropriate guidance has been developed and tested”.

Therefore, additional field studies were evaluated to show the relevance of aged sorption in the field. The evaluation was mainly based on the comparison of observed concentration depth profiles versus FOCUS-PEARL simulations with and without consideration of aged sorption. Where possible, site-specific aged sorption parameters from laboratory studies with the respective field soils were used. The depth to which 95% of the residues are found was defined as metric for comparison. It was found that in all studies and sites the simulated soil profiles without aged sorption significantly overpredict the leaching of the substances. When considering aged sorption in the simulation the leaching was mostly still overpredicted but to a lesser extent which confirms the relevance of aged sorption in the field. In addition, the increase of apparent  $K_d$ -values from field and laboratory aged sorption studies with the same soils were compared with the result that the effects of aged sorption in field soils are similar to what is observed in laboratory studies.

### **3.17.V Measuring, Modelling, and Monitoring the Environmental Fate and Exposure of Pesticides**

#### **3.17.V-01 Microextraction and Chromatographic Determination of Selected Carbamate Pesticides in Soil Samples**

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Pesticides are generally applied to agricultural crops for protection but their residues present a potential risk to non-target organisms, affecting aquatic ecosystems and posing risks to human health. Organophosphates and carbamates impair nerve transmission in insects and poses even higher human health risks if left undetected. Recent advances have shown that these pesticides can be extracted and preconcentrated from aqueous samples by liquid-phase micro-extraction (LPME), solid phase extraction (SPE) and headspace solid phase micro extraction (HS-SPME), while supercritical fluid extraction (SFE) and pressurised fluid extraction (PFE) can be employed for sediments. This study has shown that improved extraction allows for sensitive detection of carbamates in soil and fruit samples.

The study involves the testing of a rapid, reliable, and low-solvent extraction procedure for selected carbamate pesticides of carbaryl, carbofuran, and methomyl from soil and fruit samples. It involves an investigation to determine which solvents to use, extraction optimisation, and how much of the applied carbamates are found in the fruit, compared to the soil in the same geographical location.

Preliminary results have shown that the concentrations are accumulated in the soil samples and have seasonal trends.

#### **3.17.V-02 Organic Matter Reduces the Sorption Capacity of the Pesticide Fipronil onto Polyethylene Microplastics in Surface Water**

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Microplastics are ubiquitous contaminants in the environment. In water, they can sorb hydrophobic contaminants, leading to their accumulation and transport in the aquatic environment consequently exposing organisms to their toxic effects. Several pesticides are hydrophobic and persistent; therefore, they have great potential for sorption onto microplastics. Fipronil, a pesticide detected in several water bodies in Brazil, is susceptible to transformations such as reduction, oxidation, hydrolysis, and photolysis. Fipronil-sulfone and fipronil-sulfide are major biodegradation products of fipronil. We aimed to assess the interactions of fipronil, fipronil-sulfone, and fipronil-sulfide with polyethylene (PE) microplastics in ultrapure water and river water to evaluate the sorption capacity of the contaminants onto PE. Kinetics experiments were conducted in 10-mL glass test tubes containing 4 mL of

contaminants dissolved in ultrapure or river water (1000, 250, and 800 µg/L of fipronil, fipronil-sulfone, and fipronil-sulfide, respectively) and 20 mg of PE microplastics (average particle size = 107 µm). The tubes were kept in a rotating shaker at 40 rpm for different contact times. Liquid samples were analyzed by LC-MS/MS. In ultrapure water, fipronil and its degradation products were greatly sorbed by PE microplastics. At equilibrium (48 h), we observed sorption of 86, 86, and 76% of the fipronil, fipronil-sulfone, and fipronil-sulfide initially present in the solution, respectively. For the three compounds, the sorption was described as a pseudo-second-order reaction. No sorption was observed in the controls without microplastics. In river water, the sorption capacity of PE microplastics was substantially reduced. Sorption was slightly higher in the treatment containing fipronil and microplastics (46%) than in the treatment without microplastics (36%). For fipronil-sulfone and fipronil-sulfide, no difference between the treatments with and without microplastics was observed. Experiments with added humic acids showed that high concentrations of dissolved organic matter in the water lead to lower sorption capacity. Our results indicate that experiments conducted in ultrapure water might not represent the complexity of sorption reactions in natural environments. Therefore, further studies are needed to understand the impact of microplastics on the retention and transport of contaminants in freshwater bodies and their effects on aquatic organisms.

### **3.18 One Health Next Generation Wastewater Management and Reuse: The Role of Non-target and Retrospective Analysis, Bioassays, and Wastewater Surveillance**

#### **3.18.T-01 Microbiota as Drivers and Markers of Water and Soil Pollution and Recovery**

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Microbia are the universal drivers of all geochemical cycles, on which all the Biosphere depends. On a smaller scale, microbial composition has been long used as a marker of anthropogenic impact, mainly by the identification and quantification of human microbial pathogens and commensals. The current ability to describe whole microbiomes by high throughput DNA sequencing opened whole new ways of monitoring alterations, anthropogenic or not, on the ecosystems. In turn, microbial metabolism and growth are central in the process of depuration of water bodies, polluted soils, and other potentially harmful environments. We observed in different field studies how the microbiome was changed by the presence of anthropogenic pollution and how its reversal took place under distinct scenarios. These processes applied to both biological (pathogens, antibiotic resistance, gut bacteria) and chemical hazards (CECs). We propose microbiota analysis as a way to monitor the pollution of impacted sites and, at the same time, a guide to develop treatment schemes favoring the displacement of gut-related, copiotrophic bacteria by oligotrophic or mesotrophic microbiomes. We found this mechanism operating in processes as water depuration through managed aquifers, manure composting, and soil re-naturalization after organic amendment. Our results also suggest that the lack of such a microbiome substitution in current water treatment processes is responsible for their failure in eliminating many relevant hazards, including Antibiotic Resistant Genes (ARGs) and CECs.

#### **3.18.T-02 Environmental proteomics in wastewater-based monitoring. Determination of large biomolecules as biomarkers of population health and activities.**

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The analysis by mass spectrometry of small molecules in wastewater is a widely adopted approach for population monitoring by governmental institutions. Typical applications are for example monitoring the consumption of tobacco, alcohol, or drugs by a community (and, more recently, using PCR, to evaluate COVID prevalence by measuring virus RNA in wastewater. Current development of mass spectrometry allows extending this approach to large molecules such as proteins opening the window to the monitorization of human health biomarkers already known in clinics.

Previously, we reported for the first time on the peptide and protein components absorbed in a support submerged in wastewater at a WWTP. We detected proteins from prokaryotic to higher eukaryotic organisms, covering plant, animal, and human proteomes as well. We were able to identify not only major components like albumins, keratins, etc., but also less abundant ones (ie, S100A8, uromodulin, defensins, etc.) that are known as disease biomarkers.

We study the composition of the soluble fraction of wastewater collected at the entrance of 10 different WWTP in Catalonia at three different times of the year. The objectives are (a) the deep proteomic characterization of the wastewater proteome (b) the comparison of the protein profile of wastewater derived from 10 different places in Catalonia covering a range of different sizes and influent characteristics (relative contribution of domestic and industrial load, and lastly (c) to contribute to the improvement of the WWTPs operation and management.

We identified near 1000 proteins from human and other species. The most abundant proteins include human pancreatic enzymes and albumin from different livestock animals. Our results show how the protein profile in each of these municipalities reflects their agricultural activity and its potential application to pest control or to monitor the levels and source of animal tissues dumped by the industry. Also, the proteome of influent and effluent of some of this WWTP were compared. The results indicated that almost all the proteins are eliminated in the treatment plant.

#### **3.18.T-03 Operational Invertebrate Behaviour Videotracking for Real-Time Wastewater Surveillance and Management**

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As availability of water resources decreases, the apparition of new contaminants of concern (CEC) identified in water networks continues to increase. Current wastewater monitoring in treatment plants relies on interval-based grab sampling measurements for a select few chemicals or for punctual effluent toxicity assessment using a restricted choice of bioassays. As this strategy is known to omit at large portion of micro-pollutants and contaminants of emerging concern (CECs), innovative approaches to wastewater management are essential to both avoid potentially detrimental impact of largely unknown CEC discharge in aquatic ecosystems and improve efficiency of urban water re-use.

In previous work using the biomonitoring apparatus ToxMate, whereby the video-tracking of locomotor behaviour of bio-indicator invertebrates (*Gammarus*, *Radix*, and *Erpobdella*) generates positional data in the on-line wastewater multi-species monitoring device, we showed the bio-test method to be effective in non-targeted effect-based screening of micro pollutant presence (including little known emerging contaminants). In continued research we illustrate results from long term surveillance in WWTPs to optimise operation, where the characterization of the effluents in real-time for the multispecies approach alerts operators and allows strategic sampling and effluents to better understand treatment efficiency. Finally, continued laboratory testing has led to the accumulation of behavioural reaction analysis for over 80 micropollutants. Definition of multi-species behavioural fingerprints to characterise causes for temporal variability in CEC discharge is presented using functional data analysis (FDA) and artificial intelligence.

Continuous surveillance not-only identifies critical moments but has incorporated timely sampling techniques to validate reactions. Coupled with behavioural fingerprinting, these moments may help adapt wastewater management strategy in real-time. Thus, the potential is not only limited to the improvement of aquatic environmental quality through wastewater management, but could also provide concrete indicators of the suitability of re-use in various domains such as urban, agricultural and leisure use.

### 3.18.T-04 Nutrient Recycling from Urine and Faeces: Problematic of Micropollutants

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Most nutrients from human metabolism are excreted with urine. Therefore, urine collection and treatment allows production of a valuable fertilizer and promotes sanitation in areas where no sewer-based sanitation is available. To treat the urine, nitrification followed by distillation is the best tested technology. However, pharmaceuticals are not well removed during storage or biological treatment of urine. Therefore, we aimed to test the removal of pharmaceuticals from nitrified urine by granular activated carbon (GAC) in a flow-through filter to evaluate the capacity until breakthrough at different hydraulic retention times. GAC can be used in convenient filter beds and has, compared to powdered activated carbon, the additional benefit that it can be regenerated and reused. Two GAC columns with 2 m height and 1.3 kg material were run with nitrified urine and had valves at several heights to allow sampling at empty bed contact times (EBCTs) of 25-115 min. Complete removal of 11 investigated pharmaceuticals from nitrified urine was achieved for up to 660 bed volumes at an empty bed contact time (EBCT) of 70 min. The recommended EBCT for urine treatment (70 min) is much longer than what is suggested for the treatment of municipal wastewater treatment plant effluents ( $\geq 20$  min), and the amount of treated bed volumes until breakthrough is shorted. This is probably due to the approximately twenty times higher DOC content in nitrified urine compared to municipal wastewater. These results have led to the production of a urine fertilizer that is authorized for use on vegetables and flowers in Switzerland. Beside urine, human faecal compost from commercial public composting toilets was investigated for its content of pharmaceuticals. Currently, the use of faecal compost from composting toilets for land application is prohibited in Switzerland.

### 3.18.T-05 Development of a Reusable Super-biochar With Antibiotics Degradation Capacity

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Antibiotics-resistant gene development has been linked directly to the presence of antibiotics in our environment. The various reports on antibiotics resistance indicate that the ubiquitous nature of these antibiotics in the environment will eventually lead to an outbreak of total drug resistance diseases if adequate precaution is not taken. Several methods for the removal of various antibiotic contaminants have been proposed. These approaches include advanced chemical oxidation, membrane filtration, adsorption using activated charcoal, and recently biochar. Among these techniques, biochar-based adsorbent for antibiotics removal provides a low-cost and environmentally friendly approach. There has been development of various biochar-based remediation systems for efficient removal of a number of antibiotics from contaminated water. However, biochar is known to remove the antibiotics by absorption mechanism and could not mineralize the antibiotics. The inability of biochar to degrade or mineralize adsorbed antibiotics may lead to the production of secondary pollutants. Also, the desorption of the antibiotics to regenerate the biochar often results in additional cost, which in effect limits the cost advantage of using biochar-based antibiotics adsorbents.

Since its discovery in 1990, various advanced chemical oxidation processes (ACOP) have shown the potential to oxidize and mineralize various persistent organic pollutants (POPs), such as antibiotics, to an acceptable level without producing toxic by-products. However, ACOP is expensive due to the requirement of an activation process. Developing systems that could allow for efficient and reusable activation catalysts for ACOP would be beneficial. Therefore, the research aims to develop a low-cost and highly efficient treatment system that combines the benefit of biochar-based adsorbents with a persulphate-based chemical

oxidation process for the complete removal and mineralization of antibiotics contaminants. Overall the project aims to reduce the cost of antibiotics remediation without the generation of secondary pollutants, with a view to making this technology available to low-income countries with water scarcity.

### **3.18.P One Health Next Generation Wastewater Management and Reuse: The Role of Non-target and Retrospective Analysis, Bioassays, and Wastewater Surveillance**

#### **3.18.P-Tu302 High Resolution Mass Spectrometry for the Analysis of Contaminants and Metabolites from Treated Wastewater: The Case Study of Saudi Arabia**

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Anthropogenic pollution can assume different forms and impacts on all environmental compartments. Particularly, a high number of contaminants and their metabolites and/or degradation products have been widely reported in the environment. Chemical monitoring based on the determination of target compounds fails to consider most known environmental micropollutants, not to mention the unexpected and unknown chemicals. Currently, high-resolution mass spectrometry (HRMS) data with wide-scope target, suspect, and non-target screening is recommended.

The purpose of this study was to investigate and compare plants growing in two Saudi Arabia areas, one corresponding to the South Riyadh (large industrial city, >10 million inhabitants, intense traffic) and the other to Abha (small city, < 760000 inhabitants, near a natural park), as bioindicators and biomarkers of anthropic pollution. To this end, ThermoScientific™ Orbitrap Exploris™ 120 mass spectrometer and Thermo Scientific™ Vanquish™ UHPLC coupled with Thermo Scientific™ Tracefinder™ and Compound Discoverer™ software, which achieved quantification as well as data interpretation and structure elucidation were used.

Various MS<sup>2</sup> modes were investigated, including the combination of full scan mass range at a resolution of 60000 followed by MS<sup>2</sup> with 4 precursor isolation windows for the full MS range or S<sup>2</sup> (target acquisition) against a mass list. This approach facilitated identification without unnecessary increase in the cycle time. Using this system, several unexpected environmental contaminants were identified showing the possibilities of the application of mass spectrometry.

Explores 120 MS high sensitivity, high dynamic range, and high mass accuracy enabled accurate identification of trace level contaminants in complex environmental matrices, such as water, sediment and food.

#### **3.18.P-Tu303 Polystyrene Nanoplastics Removal from Urban Wastewater by Aerobic Membrane Bioreactor**

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Micro- and nano-plastics as emerging contaminants have become an increasing environmental concern given their persistent and ecotoxicological effects on the aquatic environment. Although the retention capacity of identified microplastics in wastewater treatment plants (WWTPs) ranges between 98 % to 99 %, WWTPs are still considered the main pathway for microplastics to get into the aquatic environment. In this context, membrane-based advanced technologies for wastewater treatment, such as membrane bioreactors (MBRs) have been demonstrated to reach a removal capacity of 99.9 % of microplastics (MPs). However, the removal of nanoplastics (NPs; < 1 µm) has been scarcely investigated. Thus, the present work aimed to evaluate the removal capacity of nano-plastics by an aerobic MBR (aMBR) of submerged-membrane configuration.

To evaluate the removal capacity of NPs (polystyrene (PS) of 120 nm sizes) by aMBR, two systems were employed (working volume of 30 L). One system was used as control (aMBR-Control) (i.e., without PS addition), and the other with PS nanospheres spiked (aMBR-PS). The performance of three different membranes (microfiltration, ultrafiltration, and recycled ultrafiltration membranes) was compared and their surface morphologies were evaluated. The quality analysis of the aMBR permeates and the characterization of the mixed liquor (ML) properties (i.e., physical-chemical parameters, microbial community compositions, and floc micro- and macroscopy characteristics) were conducted. In addition, the PS quantification for ML and permeate samples by pyrolysis gas-chromatography mass was examined. Preliminary results showed an overall efficient performance of the technology for NPs treatment in terms of permeate quality. However, the membrane resistances were highly increased due to the internal pore blocking under NPs. Indeed, the total resistance of the microfiltration membranes evaluated in the aMBR-PS was 4.1 times more than the submerged membrane in aMBR-Control at the end of the 38-day experiment. Regarding the influence of PS nanospheres on the ML, analysis of the floc characteristics, microbial community compositions, and soluble microbial products are under current study. By the completion of the current research, our findings could offer valid insight into the NPs removal capacity of aMBR technology, sludge floc characteristics, and membrane fouling behavior.

#### **3.18.P-Tu304 Minimization of environmental impact of protein containing wastewaters by membrane treatment**

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The dairy industry generates a large amount of protein-containing wastewater, which is characterised by high values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Dumping such wastewater into water bodies without proper treatment may cause serious environmental harm.

Currently, membranes are utilised for various water treatment and desalination applications, including the treatment of protein-containing wastewater; however, developing innovative membranes with improved properties is necessary to handle membrane fouling/scaling concerns better and enhance the chemical/mechanical properties of membranes.

We prepared novel polyethersulfone (PES) ultrafiltration membranes incorporated with carrageenan, which is a natural



polysaccharide extracted from seaweed. We employed these membranes to treat both model bovine serum albumin (BSA) solutions and real protein-containing wastewaters from the dairy industry. The fabricated membranes were characterised, including water contact angle and zeta potential analysis, SEM, total porosity, and pore size measurements. The properties of the prepared membranes were compared against neat PES and commercial ultrafiltration membranes. It was found that the carrageenan incorporation in the PES polymer matrix enhances the hydrophilicity, surface charge and compaction resistance of PES/carrageenan membranes. The prepared membranes underwent filtration and fouling tests using bovine serum albumin (BSA) solutions. It was found that the PES/carrageenan membranes possessed higher resistance to BSA fouling than the neat PES membranes, with a notable decrease in irreversible fouling while maintaining BSA rejection above 96%. Finally, PES/carrageenan membranes successfully reduced COD and BOD values when employed for the treatment of total protein-containing wastewater from the dairy industry in Qatar.

### **3.18.P-Tu305 Removal of pharmaceuticals and personal care products from hospital effluents by biochars produced by cookstoves**

**Brigitte Mukarunyana**, *Chemistry, Umea University, Sweden*

The occurrence of pharmaceuticals and personal care products (PPCPs) in water systems can affect aquatic life. Wastewaters including those discharged by hospitals can contribute to the increase of PPCPs in the environment. Conventional methods applied in most wastewater treatment plants have been used to remove PPCPs from wastewaters, however, different studies showed that those methods are not efficient in terms of PPCPs removal. To mitigate that issue, different carbonaceous adsorbents like activated carbon have been used to treat PPCPs but the activated carbon is costly therefore, it is challenging to use, especially in countries with low incomes. Biochars could thus be an alternative because the raw materials used to produce them are locally sourced. Various studies have been performed to investigate the efficiency of biochars in treating wastewater, however, the results are not yet coherent, in addition, biochars have different characteristics depending on the feedstock from which they are made and the conditions applied during their production. Further research on biochars as adsorbents and experimental studies are of high importance in the wastewater treatment domain. This study aimed to investigate the ability of biochars produced by cookstoves to remove PPCPs from hospital wastewater.

Wastewater samples were taken at Rwanda's University Teaching Hospital of Kigali (CHUK). The biochars produced by 3 different cookstoves using 3 feedstocks have been characterized using DRIFTS, Raman spectroscopy, XPS and BET. Wastewater samples have been analyzed using LC-MS/MS method before and after being treated by biochars. The results from XPS analysis showed a high % of carbon for all biochars, this observation was supported by the abundance of C-H peaks for aliphatic and aromatic functional groups indicated by DRIFTS and Raman spectra. 28 PPCPs have been detected and quantified with the abundance of antibiotics. PPCPs have been removed at different degrees depending on the type of biochar. The removal rates (RR) ranged between 0.2 and 97.7% and about 52% of PPCPs were removed at a RR  $\geq 70\%$  by the top 2 best biochars. Results showed that the surface area of biochars might not be the main driving force of PPCPs removal. Findings will contribute to further studies on biochars especially on characterizations to investigate the driving forces of PPCPs removals.

### **3.18.P-Tu306 Antiviral Performance of Metal Foam in Water Disinfection**

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It is well known that waterborne bacteria is a significant health concern in water safety, which can cause immediate illness. However, the presence and health impact of waterborne viruses are less appreciated. The potential health risks caused by viruses shed in the wastewater are undervalued, and effective virus disinfection techniques are urgently needed to eliminate the risks of viruses. A broad spectrum, effective, mobile, and economically feasible point-of-use water treatment system is urgently desired in developing or remote households and emergency relief settings.

This study investigated the antiviral properties of the micro-structured copper foam in the water environment. The bacteriophage MS2 was adopted as a surrogate for the human virus to predict the interaction and behavior in a water environment. The experiment was carried out under both a virus sole-existence in water environment, and host cell/virus co-existence in a water environment to study the life cycle inhibition and reveal which stage of the virus replication cycle could be blocked by copper foam. The effect of retention time, host cell presence, and virus load were considered, and the potential mechanism is discussed. The result demonstrated that the micro-structured metal foam cannot effectively inactivate viruses in a virus-only environment but can effectively inactivate viruses in a virus/host cell co-exist environment. With the presence of host cell *E. coli*, the killing rate of copper foam was significantly improved, reaching 92% after two hours. Comparatively, the killing rate of MS2 with host cells and with no copper foam treatment was only 15.9% at the end of the experiment, which is explained by the replication process of the MS2 with host cell supply. The hypothesis of the mechanisms is that the virus is attached to its host cell using virus attachment protein to facilitate its docking onto the host cell. Furthermore, the initial contamination level and the attachment status between viruses and host cells play an important role in the survivability of viruses in water and the antiviral performance of the copper foam. The copper foam demonstrated higher inactivation rates under low contamination levels and also higher inactivation rates on viruses at the latent period.

The study validates the hypothesis that metal foam can have effective virucidal performance, and is valuable for further research on antiviral applications of microscale metal materials.

### **3.18.P-Tu307 Toxicity of Carbamazepine after Plasma Oxidative Degradation in Freshwater Green Algae (*Raphidocelis subcapitata*)**

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## Belgium

The anticonvulsant drug carbamazepine (CBZ) is continuously discharged into natural water, as it is not well removed by conventional wastewater treatment plants, which may pose a concern to the aquatic ecosystem. Advanced oxidation processes (AOPs) have been proven to effectively remove CBZ and other pollutants. Plasma oxidative degradation (POD) is being thoroughly researched because of its fast, feasible, and low environmental impact characteristics. A gas phase surface di-electric barrier discharge plasma system is used, where reactive oxidative species are generated in situ and brought into contact with the contaminants via a carrier gas to degrade them. However, unwanted toxic transformation products (TPs) could be generated. In this study, the toxicity of CBZ TPs after POD was examined using freshwater green algae (*Raphidocelis subcapitata*). The POD experiment was conducted using 10 mg/L CBZ with an applied voltage of 14-15 kV, and the samples were collected at different time points, including 0 min (without POD treatment), 12 min, 22 min, and 30 min. After 12 min of degradation, all CBZ was degraded, and mixtures of TPs were present. The results revealed that the samples after treatment with POD induced algae toxicity at all time points. However, no toxicity was observed in the sample before POD treatment. The algae toxicity increased after a longer POD treatment time. For the longest degradation time, the lowest EC10 is observed at 11.9% of the original sample. Based on our observation, the oxidation of CBZ was likely through a reaction with ozone-reactive species generated during plasma discharge. We hypothesize that CBZ ozonation byproducts, including carbamazepine-(10,11)-epoxide and 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD), could be the main effect drivers due to the high toxicity according to ECOSAR for algae toxicity. Further chemical analysis will be performed to confirm and link CBZ TPs with the observed toxicity. Our study highlights the importance of toxicity evaluation before discharging AOP-treated (waste)water streams into the environment due to possible toxic transformation products.

### 3.18.P-Tu308 Stop Wasting Wastewater! Wastewater Quality Assessment After Advanced Treatment for Potential Reuse.

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Water reuse applications, such as the reuse of treated wastewater (TWW), are attracting global interest. This is being accelerated by increasingly frequent freshwater shortages, as water resources are under increased pressure due to rising populations and climate change. However, conventional wastewater treatment plants (WWTPs) do not focus on the abundance of anthropogenic chemicals potentially present in the water cycle, resulting in the fact that TWW generally contains micropollutants and their transformation products. Consequently, in circular systems, this increases the potential for the accumulation of hazardous and persistent compounds and may lead to negative consequences for human and environmental health. Thus, to utilize the true potential of water reuse and achieve acceptable risks, these pollutants must be removed from the water prior to its reuse.

In Wervershoof, the Netherlands, a pilot plant was installed on the local WWTP to study the potential reuse of TWW for potable to non-potable reuse applications. The WWTP effluent served as the intake for the pilot plant and was treated by a variety of additional treatment techniques. In summary, these consisted of ozonation, later replaced by advanced oxidation, ceramic membrane filtration, and activated carbon. Over the summer of 2022, a total of 90 grab samples were taken in triplicates. Sampling points included the pilot intake and directly after the aforementioned treatment steps. The samples were supplemented by field blanks and all samples were analysed for common micropollutants covering pharmaceuticals, personal care products, pesticides, and industrial chemicals. After solid-phase extraction (Oasis HLB), the extracts were analysed by LC-HRMS and effect-based tools such as *in vitro* CALUX bioassays. This approach not only covers known target substances but also unknown compounds, and the overall mixture toxicity is assessed.

This poster presents the first results of this study and gives indications about the potential water reuse applications of TWW that could be achieved by the respective treatments of the Wervershoof pilot plant. Future work will also include suspect and non-target screening, as well as more intensive bioanalysis. Our results will help water authorities develop an improved understanding of the linkage between appropriate water treatment techniques for potential water reuse applications, suitable monitoring techniques, and of potential environmental and human health risks.

### 3.18.P-Tu309 Comparison of ozone- and UV-based technologies for trace organic compounds removal from wastewater

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Trace organic pollutants (TOCs; aka micropollutants [MPs], contaminants of emerging concern [CECs], etc.) such as pharmaceuticals and personal care products, hormones and pesticides, as well as their transformation products reach wastewater treatment plants (WWTPs) on a continuous basis. Conventional wastewater technologies are not designed to deal with this kind of pollution, and therefore many organic compounds leave treatment facilities after being only partially removed/transformed. As treatment wastewater is usually discharged into surface water bodies or the ocean, WWTP effluents can be considered to be one of the main sources for TOCs into the aquatic environment.

Chemical oxidation processes can be effective technologies for the removal of micropollutants from water and wastewater. Among all available systems, the homogeneous ozone- and UV-based oxidation technologies, both based on the action of hydroxyl radical ( $\bullet\text{OH}$ ), are especially promising due to their potential action against different classes of chemicals and their relative maturity/potential implementability at full-scale. However, a rigorous comparison of processes considering techno-economic and environmental criteria is required to decide which technology is most suitable in terms of pollutant destruction efficiency, running costs and associated impacts.

In this work, a range of ozonation,  $O_3/H_2O_2$ ,  $UV/H_2O_2$ ,  $UV/persulfate$  and  $UV/Cl_2$  processes were compared in the abatement of 20 organic pollutants with different structure/physicochemical properties, spiked at trace level (1  $\mu g/L$ ) to 5 different wastewater effluents collected in the Barcelona metropolitan area and presenting different physicochemical characteristics (total organic carbon [TOC]: 5.5–18.5 mg C/L; alkalinity: 67–360 mg  $CaCO_3/L$ ; pH: 7.4–8.2;  $NO_2^-$ : 0.05–1.6 mg N/L). Oxidation experiments were conducted at lab-scale level simulating actual operational conditions regarding both oxidant doses and exposure to UV light. Samples of treated effluents were collected and subjected to solid-phase extraction (SPE) followed by analyses by HPLC-MS/MS. The economical and environmental evaluation of each process was based, respectively, on the capital/operational costs and life cycle impacts associated to the treatment of a given volume of wastewater effluent to reach particular micropollutant abatement levels.

### 3.18.P-Tu310 Nature-Based Solutions as a Pretreatment of Photo-Fenton at Natural pH to Enhance the Removal of Contaminants of Emerging Concern

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The increasing concern on the presence of contaminants of emerging concern (CECs) in water bodies and their potential risk for human health and ecosystems has motivated the development of new suitable technologies to eliminate diverse type of CECs. Advanced Oxidation Processes (AOPs) have proven to be efficient in the abatement of a wide amount of recalcitrant compounds. However, elevated costs and energy demand for their implementation and maintenance are linked to these processes. In this regard, Nature-based Solutions (NbS) such as constructed wetlands (CW) have emerged as a green alternative since they have also demonstrated their potential in the removal of CECs. Nevertheless, this technology also presents disadvantages which could limit its implementation at full scale: requirement a larger area footprint and long operation treatment times. In this study hybrid system composed by CW coupled to photo-Fenton process (type of AOP) at natural pH was investigated to solve the individual limitations of using two technologies separately.

Therefore, the purpose of this study was to evaluate the performance of four CW (lab-scale, 1.5 L) planted with *Phragmites australis* or *Cyperus haspan* and two operational modes: with and without recirculation on the removal of 17 micropollutants at 1  $\mu g/L$  in secondary wastewater effluent. Additionally, the potential of CW as an effluent pretreatment for solar photo-Fenton at natural pH using DTPA-Fe (III) was also investigated.

Regarding the CW unit, obvious differences were shown when two modes of operation were compared. Highest removals of CECs and dissolved organic carbon (DOC) were found in CWs with recirculation. An average of 8 cycles revealed the elimination of 50% of DOC and 65% CECs in two CW with recirculation, while only 35 and 40%, of DOC and CECs, respectively, were achieved in CWs without recirculation.

Concerning the hybrid system, the results disclosed a reduction of more than 4 times of operation time when wastewater was pretreated with CW with recirculation. For instance, total metronidazole removal was achieved at 30 minutes while without any pretreatment only 80% removal was observed after 2 h of treatment. In the pretreatments without recirculation the improvement was lower (total metronidazole removal was found at 2 h). This fact highlights the potential of recirculated constructed wetlands coupled with solar photo-Fenton as efficient and ecofriendly solution for the possible reuse of wastewater.

### 3.18.P-Tu311 Wastewater based epidemiology used for monitoring the trends of covid-19 epidemic in the Czech Republic

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Shortly after the outbreak of the coronavirus, it was found that its genomic fragments are secreted not only by the respiratory system but also by the digestive and excretory tracts. In this way, they enter wastewater, sewage systems and wastewater treatment plants. This principle has been used in many countries to test the possibility of objective monitoring of the incidence of the disease in the population.

Our research began in 2020 by evaluating the possibilities to use Wastewater Based Epidemiology (WBE) approach in the Czech Republic by monitoring the occurrence of SARS-CoV-2 RNA in untreated water from wastewater treatment plants (WWTP). Since the beginning of monitoring, 66 WWTPs of various categories (most of 10s–100s of thousands inhabitants served) have been monitored in various sampling regimes.

RNA concentration was performed by direct flocculation. It was followed by RNA isolation using QIAamp Viral RNA Mini Kit. Quantitative detection of SARS-CoV-2 RNA in wastewater samples was performed using RT-qPCR. The amount of genomic equivalents of viral RNA was compared with numbers of positively tested people connected to the relevant WWTP.

The results, in most cases, showed correlation between these data. The chosen method captured the trends of decrease and increase of all epidemic waves. The method seems to be very promising in systematic monitoring as an early warning tool and in helping to ensure that appropriate measures are taken.

### 3.18.P-Tu312 Application of Nanofiltration Processes for Removal of Active Pharmaceutical Ingredients in Hospital Wastewater Treatment

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In wastewater treatment, conventional processes are not suited for efficient removal of POPs and emerging pollutants, which can pose a threat to aquatic wildlife and human healthcare. To mitigate their release to the environment, various technologies were investigated in recent years, with a focus on adsorption, oxidation and membrane filtration processes. While nanofiltration

processes are more expensive, they can play a part in closing the loop of the water economy, as nanofiltration processes generate high quality effluent due to complete rejection of pathogens and high rejection of extracellular resistance genes.

To investigate the capability of nanofiltration processes for removal of active pharmaceutical ingredients in hospital wastewater, flat-sheet membranes with different rejection and permeability characteristics are screened with a laboratory-scale membrane filtration unit. This work aims at selecting suitable nanofiltration membranes and provides the basis for further process optimization and pilot-scale studies. Emerging from the interdisciplinary PharmCycle project at the Hamburg University of Applied Sciences, this research work is part of a cooperation project coordinated and financed by HAMBURG WASSER. Accompanied by the Hamburg University of Applied Sciences and the University Medical Center Hamburg-Eppendorf, micropollutant removal efficiencies of various stand-alone and hybrid processes are evaluated for future applications in municipal wastewater treatment.

### **3.18.P-Tu313 Microplastics removals in two drinking water treatment plants with different treatment technology supplying Barcelona city (Spain)**

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Microplastics (MPs) are frequently ingested by humans, demonstrated by their presence in human faeces in several studies. One of the routes for human MPs consumption is drinking water, thus some studies have reported MPs in bottled water and tap water, however there are not enough studies about the effect of technology of drinking water treatment plant (DWTP) in MPs removal efficiency to provide MPs-free water. The aim of this study was the identification of MPs in large volumes of drinking water derived from two different drinking water technologies in a densely populated region of Spain, as well as the determination of the effect of water conditioning treatment. Sampling included raw water inflow and outflow of different treatment units of both, conventional DWTP and desalination plant (DP). Sampling strategy consisted in a filtration of large volumes of water with an in-line hermetic stainless steel filter holder containing a PTFE filter (10 µm pore size) connected to each treatment unit's representative pipes by 8 to 9 h. Sample treatment consists of oxidation with Fenton's reagent or hydrogen peroxide 35% according to high colour and organic matter content. To remove inorganic matter, density separation was applied using zinc chloride (density: 1.8 g/mL). Visual identification was performed with a stereoscopic microscope LEICA MZ10 coupled to FLEXACAM C1 camera and Olympus CX41 optical microscope. All particles detected  $\geq 20$  µm were picked up and analyzed by µFTIR on calcium fluoride slides and ATR-FTIR spectroscopic techniques. To avoid the loss of MPs by missing particles recruitment from 20 to 50 µm, filters were washed and filtered on siliceous filters pore size 5–6 µm wide. Both, DWTP and DP, presented high MPs removal efficiency set at 98.3 and 92.6%, respectively. Units involving addition of chemical reagents, such as coagulation-flocculation-air flotation in DP and remineralization units, presented a decrease of removal efficiency. In the reverse osmosis process, high removal of up to 99.9% was observed. The fibres were predominant in outflow of first treatment units, but fragments were in the last units. Assuming a water consumption per capita of around 1.0 L/day and all tap water are coming from DWTP and DP without extra incorporation of MPs in distribution pipelines system, the annual intake of MPs through tap water was set at 27 and 44 MPs/person/year respectively.

### **3.18.V One Health Next Generation Wastewater Management and Reuse: The Role of Non-target and Retrospective Analysis, Bioassays, and Wastewater Surveillance**

#### **3.18.V-01 Adsorption of Congo Red and Methylene Blue on Zinc Oxide Nano-particles and Zinc Oxide Nano-particle modified Cassia fistula Seed pod**

*Alimoh Helen Alabi, University of Ibadan, Nigeria*

Pollution of water bodies by highly carcinogenic and hazardous chemical substances such as dyes which is detrimental to human-health and the environment is now a major concern. Adsorption of dyes by low-cost adsorbents have been reported, but with a few shortcomings. This study therefore aims to use zinc oxide nanoparticles (ZNP) and zinc oxide nanoparticle modified *Cassia Fistula* (ZNPMCF) pods as adsorbents for the removal of Congo Red (CR) and Methylene Blue (MB) from aqueous solution. The ZNP and ZNPMCF was prepared using a simple precipitation method. Fourier Transformed-Infrared (FT-IR) was used to determine the functional groups on the adsorbent surface. ZNP and ZNPMCF were used as adsorbents for the removal of CR and MB. The effects of various process parameters i.e., adsorbent dosage, pH, contact time, initial concentration and temperature were investigated for each adsorbent for both CR and MB from aqueous solutions. The adsorption data was fitted using Langmuir, Freundlich, Temkin, Sips and Redlich-Peterson adsorption isotherm. Also, the Pseudo-first order, pseudo-second order and the intra-particle diffusion kinetic models were used to fit the kinetic data.

Both adsorbents demonstrated relative dye removal efficiencies with that of ZNPMCF exerting a higher adsorption for CR. CR was found to adsorb well onto ZNP and ZNPMCF at initial concentrations ranging from 20 to 70 ppm, with 98.87% at high concentration. The Redlich-Peterson model was found suitable for describing the equilibrium data for the removal of CR by ZNP and Temkin model for ZNPMCF, while the Freundlich model was best for fitting the adsorption data of MB by both adsorbents. The pseudo-second order kinetic model was also the best fit for describing the kinetics of adsorption of CR and MB on both adsorbents is the pseudo-second order kinetic model. Intra-particle modelling revealed that intra-particle diffusion does not control the dye sorption process and is not the rate limiting step.

It was concluded that the ZNPMCF is a promising adsorbent that is viable for the removal of CR dye from aqueous solutions.

### 3.18.V-02 Development of a label-free immunosensor for the detection of HAV in recycled waters

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The World Resource Institute (WRI) in recent years is alarming us about the water stress we are going through, saying that by 2025, as many as 3.5 billion people could experience water scarcity. To face this problem, ISPRA/ARPA/APPA have proposed guidelines on the reuse of purified wastewater. For many years, the potability of water or the quality of fresh seafood has been determined on the basis of the presence of coliform bacteria or different types of phages, excluding the presence of enteric viruses, as the virus of Hepatitis A (HAV). HAV (and enteric viruses in general) shows greater resistance in the environment, making it necessary to continuously monitor it. Actually, to detect HAV in aqueous media, traditional techniques such as ELISA and PCR techniques are used, but they are cost and time consuming. This work aims to develop an electrochemical sensor for the monitoring of HAV in recycled waste waters. In this regard, screen printed electrodes (SPE) drop casted with multiwalled nanotubes functionalized with carboxylic groups as enhancing material and as anchoring system were used. Upon it was built the immunologic chain through the use of the protein A and the anti-HAV which is able to recognize the HAV. The electrochemical measurements were carried out using voltammetric analysis through the use of a Palmesens4™ portable potentiostat system. The immunosensor allowed to build a calibration curve with LOD equal to  $10^{-16}$  IU/mL and a linearity range between  $10^{-16}$  and  $10^{-7}$  IU/mL, showing an RSD% of 7%. By comparing real sample results between the immunosensing platforms and PCR analysis, an important quantitative discrimination was observed. The immunosensor was able to detect HAV concentrations with higher sensitivity. In conclusion, it was possible to develop an immunosensor for HAV monitoring, able to make the detection in a short time using portable and cheap instruments, which results to be more sensitive compared to PCR technique.

### 3.18.V-03 Critical Review and Meta-Analysis of Hazardous Pollutants in Treated Wastewater for Environmental Policy of Water Reuse in Sweden

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Treated domestic wastewater is increasingly being considered a useful resource to cope with global water scarcity. However, hazardous pollutants such as metals, organic micropollutants such as pharmaceuticals, and microplastics are not completely removed in conventional municipal wastewater treatment plants and are frequently encountered in treated wastewater. Current reclaimed water quality requirements for agricultural irrigation defined by the European Union do not include these pollutants and can be an important impediment to safe reuse of treated municipal wastewater. Proposed revisions to urban wastewater treatment directive of the European Commission also promote reuse of treated wastewater and include obligation to apply quaternary treatment to urban wastewater. Therefore, it is essential to evaluate occurrence and removal of hazardous pollutants in municipal wastewater treatment plants to develop effective regulatory requirements and monitoring guidelines. The key aim of our work is to enhance the knowledge on the sustainability of wastewater reuse as a resource, focusing on the impacts of hazardous pollutants in the treated wastewaters. To this end, we compiled data and information through a systematic literature search of peer-reviewed scientific articles (by far 1785 articles retrieved through Web of Science and Scopus) as well as grey literature of Sweden (by far 49 documents retrieved through google scholar and DiVA portal). Most relevant studies from the retrieved literature will be selected using abstract screening aided by Rayyan. We will then investigate regional differences in the rate of reuse, hazardous pollutants composition in the wastewater, and key factors that contribute to these differences. A priority list of hazardous pollutants will also be compiled based on their frequency of occurrence and measured concentrations. Our study, focusing primarily on Swedish conditions, could be an example to other countries with similar climate and socio-economic conditions. Our work will also support environmental authorities in the development of future guidelines for compulsory level of treatment, keeping in view the goal of reaching a circular economy.

### 3.19.P Pet Animals' Exposure to Chemicals and Health Effects of Relevance for Humans - One Health

#### 3.19.P-Mo235 Non-Invasive Sampling to Evaluate Complex Environmental Mixtures in Pet Dogs and the Application in a Bladder Cancer Case-Control Study

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Pet dogs are considered a good comparative model for many human diseases, especially cancer. Due to their shorter lifespan, dogs have a reduced latency period between environmental exposures and cancer development and can serve as a sentinel species for identification of possible environmental risk factors linked to cancer. We established that people and their pet dogs have very similar environmental exposures by using silicone samplers as passive exposure monitors. Silicone samplers were analyzed for a suite of chemicals across many compound classes, including flame retardants, plasticizers and pesticides. Significant and positive correlations were observed between humans and dogs ( $r_s=0.38-0.96$ ,  $p<0.05$ ) for 35 of 45 chemicals measured in  $\geq 50\%$  of all samplers. Urinary biomarkers of organophosphate esters and pesticides were correlated with silicone sampler concentrations; stronger correlations were observed in dogs compared to humans. These data demonstrate that pet dogs can act as surrogates for human exposures in the home environment. In a follow-up study, we used silicone samplers to measure exposure to mixtures in a canine bladder cancer case-control study. Cases were defined as dogs with detectable urinary *BRAF* mutation levels (range 0.29–21%;  $n = 25$ ). Controls were sex, breed and age matched with no detectable *BRAF* mutation ( $n = 76$ ). The silicone samplers were analyzed using targeted and untargeted GC-MS approaches. Targeted analyses focused on 115 chemicals, of which 39 were

detected in  $\geq 50\%$  of all samples. Higher levels ( $2\text{--}3\times$ ) of 4 chemicals were measured in silicone samplers worn by cases compared to controls ( $p < 0.05$ ). While 2 more chemicals were higher ( $1.5\times$ ) in samplers worn by cases, this was not statistically significant ( $p \leq 0.09$ ). Untargeted analyses revealed a higher burden of environmental chemical exposures experienced by cases, both in the number of chemicals detected and the magnitude of exposures. Of the 1,407 features detected in  $> 50\%$  of samples, 668 were detected more in cases and 505 were detected more in controls. This study is the largest to investigate such a wide breadth of exposures associated with canine bladder cancer, the first to objectively measure exposures and the first to assess a population with subclinical disease. These data combined with the whole exome sequencing data from our cases will allow for advances in gene-environment interactions and translational medicine that may improve clinical outcomes for humans and pets.

### **3.19.P-Mo236 Contamination status and risk assessment for environmental micro-pollutants in house dust and commercial pet food collected from Japan**

**Hazuki Mizukawa**, Ehime University, Japan

A wide variety of man-made chemicals are used in the indoor environment, including plasticizers, insecticides, flame retardants, paints, pharmaceuticals, and personal care products, etc., and it is feared that indoor chemical exposure is one of the causes of allergic diseases and/or chemical hypersensitivity. It is known that humans spend 85 to 90% of their day indoors, and maintaining a healthy indoor environment is important. In Japan, more than 87% of domesticated dogs and cats are also indoors, and it is estimated that they are constantly exposed to indoor chemical substances in the same way as humans. It has been suggested that environmental chemicals may be a cause of diseases in pet animals, for example, associations between hyperthyroidism and polybrominated diphenyl ethers (PBDEs) in pet cats, and between obesity and bisphenol A exposure in pet dogs have been noted. In this study, quantitative analysis and a risk assessment of household chemicals such as insecticides and fungicides, and screening analysis of 942 environmental micro-pollutants using an automated identification and quantification system with database (AIQS) in house dust and commercial cat food collected from Japan which are important exposure media for pets. As a result, a total of 53 compounds were detected in house dust ( $n = 39$ ), including neonicotinoids, phenylpyrazole, pyrethroid insecticides, triazole fungicides, phthalates, and phosphorus flame retardants. Contaminant profiles in the dust samples seem to be affected by several factors such as region of residence, history of household drug use, and family composition. A total of 26 pesticides were detected in commercial cat foods ( $n = 31$ ), with high detection frequency and concentration of nitenpyram, chlorfenapyr, and difenoconazole. In the risk assessment based on the house dust data hazard quotient (HQ) for children, cats, and kittens exceeded 0.1 for phthalates and phosphates and exceeded 1 for fipronil, indicating considerable children and animal health risks. All the micro-pollutants analyzed in pet dog ( $n = 7$ ) and pet cat ( $n = 12$ ) serum samples were below the detection limit except only in one canine sample. Further studies and continuous monitoring based on simultaneous analysis of various micro-pollutants in dust, air, and food would be needed to clarify potential sources and exposure pathways of contaminants and to improve the indoor environment.

### **3.19.P-Mo237 Toxic Metals in Hair and Blood of Pet Dogs in Finland**

**Sarah Rosendahl**, Karperö, Finland

Toxic metals are widespread in our environment and pose a health threat to both us and our pets. Pet dogs can be exposed to toxic metals through their living environment, diet, and drinking water, but the research in this area is scarce. The burden of toxic metals can be assessed in numerous ways, for example by measuring concentrations in hair and blood. Hair analysis provides a reading of metal deposition in the cells and interstitial spaces of the hair over a 2–3-month period and has therefore been considered to reflect long-term or chronic exposure, whereas blood analysis indicates current exposure. The correlation between hair and blood has been studied a few times in dogs, but the research is still scarce. In our previous study on 50 healthy pet dogs, we showed that mercury and lead concentrations correlated between hair and blood, and that both diet type (dry food, raw food, or mixed diets) as well as specific food items (wild game, rice) were associated with toxic metal burden. However, the sample size of this study was small considering the number of variables studied. Therefore, as we have now collected more samples, the aim of this work is to repeat our previous investigations on a larger sample population. Thus, the first aim is to study the correlation between toxic metal concentrations in hair and blood, and the second aim is to search for associations between toxic metal burden and diet type or specific food items (e.g., wild game, rice). The concentrations of lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) were measured in whole blood ( $n = 120$ ) and hair ( $n = 187$ ) from client-owned pet dogs living in Finland using inductively coupled plasma mass spectrometry (ICP-MS). In addition, aluminum (Al) was assessed in hair. The correlation between hair and blood elements was controlled using Pearson's correlation and the association between diet variables and toxic metal burden was studied using generalized linear mixed models, with the selection of tested variables based on our previous studies in the area.

## **3.20 PMT/vPvM substances: Assessment, Management and Regulation**

### **3.20.T-01 Are PMOCs Less Toxic? Linking Physicochemical Compound Properties With Measured Toxicity**

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Persistent and mobile organic chemicals (PMOCs) are gaining attention as a threat to the quality of water resources. Their high mobility (i.e., polarity) and persistence lead to the presence and accumulation of PMOCs in surface water and drinking water sources. Yet, given the only recent attention to this vast group of compounds and the analytical challenges that researchers face, little is known about their toxicity. As a result, despite a growing body of environmental concentration data, risk assessment of PMOCs remains in its infancy. Therefore, the present study aimed to gain insight into the toxicity and toxic mechanisms in

relation to the physicochemical properties that determine the persistence and mobility of chemicals in the environment. It was hypothesized that the mobility of PMOCs makes them inherently less toxic than non-polar compounds. To test this, a dataset was compiled matching physicochemical data for 3360 water-relevant compounds with their measured effects in 534 unique toxicity tests from the ToxCast program. Random forest analyses identified the physicochemical properties that relate most strongly to the induced effects and linear regression analyses quantified the strength and direction of these relationships. This indeed showed that compound properties related to polarity, particularly  $K_{OW}$  and  $K_{OC}$ , are inversely related to effect concentrations, confirming that more polar compounds are, generally, less toxic. The subdivision of the dataset into particular substance groups illustrated that these relationships are more or less pronounced depending on the mode of action of chemicals, providing mechanistic insight into their toxicity. The high polarity of PMOCs may mean that they interact less with tissues, cell membranes, and receptors than their lipophilic counterparts, leading to lower compound toxicity. Although PMOCs appear to be inherently less toxic than more hydrophobic compounds, their diversity and pervasiveness in water cycles warrant further investigations into their potential threat to water quality.

### 3.20.T-02 Aqueous Leaching of Ultra-Short Chain PFAS from Fluoropolymers: Targeted and Non-targeted Analysis

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Due to the high persistence, bioaccumulation potential, and toxicity of some PFAS, the entire class is flagged as potentially concerning. Within PFAS, there are polymeric and non-polymeric PFAS, the latter of which are more thoroughly studied. Small molecule PFAS, including perfluoroalkyl carboxylic acids (PFCAs), have been measured from polymeric PFAS including side-chain fluorotelomer polymers and some fluoropolymers, but thus far, fluoropolymers have not been investigated as sources of ultra-short chain PFCAs ( $C_2$ - $C_4$ ). Fluoropolymers are widely used in laboratory settings and during our research on  $C_2$ - $C_4$  PFCAs, we have observed laboratory materials as sources of contamination. The objective of this work is thus twofold: to identify possible sources of  $C_2$ - $C_4$  PFCA laboratory contamination from fluoropolymers, and to identify additional uncharacterized sources of  $C_2$ - $C_4$  PFCAs to the environment. Fluoropolymer tubing (PTFE, PFA, FEP) and non-fluorinated polymer tubing (PVC, PP, PEEK) were tested by extraction three times into water with sonication.  $C_2$ - $C_4$  PFCAs were quantified using ion chromatography-mass spectrometry (IC-MS) with complementary non-targeted analysis of the leachate using quantitative  $^{19}F$  NMR. FEP tubing had the highest concentration of trifluoroacetic acid, exceeding 100 ng/g, followed by PFA. All tubing materials, including non-fluorinated tubing were sources of trifluoroacetic acid above our blank controls. Perfluoropropanoic acid was highest in the PFA extracts (>10 ng/g), while perfluorobutanoic acid was the highest in PTFE extracts. The heterogeneity of the PFBA measurements from PTFE (3.00–520 ng/g) suggests it may be a residual from a processing aid. NMR results aligned with IC-MS results for  $C_2$ - $C_4$  PFCAs, and also identified longer chain PFCAs, fluorotelomer compounds, and compounds containing aromatic singly-substituted fluorine atoms. The direct and unbiased nature of NMR limits additional instrument-related introduction of PFAS to the sample, strengthening the hypothesis that the PFAS are present as a result of leaching from fluoropolymers. Overall, the polymers tested had different fingerprints of  $C_2$ - $C_4$  PFCAs and other PFAS, which may be related to their synthetic processes such as processing aids, residuals, and inhibitors used. The outcome of this work allows us to better perform trace analysis in the lab and reports previously unidentified sources of these contaminants that are not currently in the global budget.

### 3.20.T-03 Efficacy of activated carbon filtration and ozonation to remove persistent and mobile substances – a case study in two full-scale wastewater treatment plants

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Persistent and mobile (PM) substances are able to spread quickly in the water cycle and where thus identified as potentially problematic for the environment and water quality. If also toxic (PMT) or very persistent and very mobile (vPvM) their regulation under REACH as substances of very high concern is foreseen. Yet, knowledge on the effectiveness of advanced wastewater treatment in removing PM-substances from WWTP effluents is limited to a few, rather well-known, chemicals. The occurrence, concentrations, and behaviour of 112 suspected and known PM-substances in advanced treatment was investigated. Two full scale wastewater treatment plants employing either powdered activated carbon (PAC) or ozonation with subsequent filtration through sand and granular activated carbon (GAC) were sampled. 73 of the 112 PM-substances analysed were detected at least once in the tertiary effluent of either wastewater treatment plant, resulting in total concentrations of 104 µg/L and 40 µg/L, respectively. While PAC was able to remove 35% of PM-substances by at least 70%, the total PM burden in the effluent was only reduced from 104 µg/L to 87 µg/L, indicating that PAC alone may not be able to reduce the PM burden in municipal wastewater significantly. Ozonation and the subsequent GAC filtration was able to reduce the PM burden in wastewater from 40 µg/L to 13 µg/L, showing a higher removal effectivity than PAC alone in this study, but also facilitating the formation of transformation products. Some PM-substances with high effluent concentrations (> 1 µg/L) could not be removed by either treatment. Among these chemicals were the scarcely studied ionic liquid constituents tetrafluoroborate and hexafluorophosphate.

### 3.20.T-04 Chemical Stripes – Visualizing Chemical Trends of the Past Influencing Today

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The number of chemicals threatening global health is rising rapidly, with increasing numbers of persistent compounds accumulating in our environment. There are alarming signals that elimination of existing and prevention of further contamination can no longer be delayed. Communicating this need for action to the scientific and non-scientific community in an understandable way poses a challenge for many researchers.

This work shows the possibility to use *chemical stripes* to help communicate this message, modifying the existing graphics of the *climate stripes* - showing the trend of global warming - and applying it to the chemical space. Specific persistent organic pollutants (POPs) including per- and polyfluoroalkyl substances (PFAS), triazines and triazoles were selected based on their presence in regulatory lists, such as the Stockholm Convention. With the use of patent data for those compounds coming from the World Intellectual Property Organization (WIPO) and the comparison to the rise of overall chemical numbers registered in databases like the Chemical Abstract Service (CAS) registry, a definite trend can be seen: Patent and overall chemical numbers are on the rise and regulations do not stop this trend. Often the drafting, discussion, and adoption of legislation takes decades, even when there is need for quick action.

The minimalistic but intuitive visualization of the *chemical stripes* helps communicate this information by indicating the evolving chemical numbers in traffic-light colours. The chemical and historical data presented, using the model of *stripes*, will be accompanied with open-source code (in progress) for others to generate their own stripes for a given set of chemicals.

The colour scheme raises awareness of the "red" state we face today, with environmental pollution impacting our health and the ecosystems we live in. All substance classes investigated so far revealed the same pattern. Overall, the aim of these graphics is to emphasize the urgent need for elimination and prevention of persistent chemicals by illustrating the exponential growth of patent and chemical numbers over time, in the hope that this will help with the identification and prioritization of harmful substance classes and to help stimulate further action, without decades of delay.

### 3.20.T-05 Occurrence of PMT and vMvP Substances in Spanish and Portuguese Transnational River Basins and Coastal Water

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There is a huge amount of chemicals included in a wide range of everyday use products with industrial or domestic applications such as pharmaceuticals, surfactants, plasticizers, UV filters, industrial additives, etc. that are still unregulated but may be candidates for future regulation according to their presence and incidence in the environment and their possible harmful effects for health. In this sense, there are several initiatives for the elaboration of pollutants' prioritization lists, based on different criteria, such as persistence (P), mobility (M), and toxicity (T) in the environment. The study of the prioritized substances' occurrence in the water cycle, considering the wastewater treatment plants (WWTP) as direct contamination sources and the surface and sea water as receiving compartments is of great importance to verify the experimental P and M of such substances. This work studied the presence of 52 contaminants of emerging concern (CECs) selected using a prioritization strategy based on P, M, and T criteria and on detection frequency (DF) data from screening studies in 93 samples collected in 2021. The 33 sampling sites included river and marine environments and WWTP located in or near national and transnational river basins and in the coast in Spain and Portugal.

The studied PMT and very mobile and very persistent (vMvP) substances presented a high DF in the national and transnational river basins being the coastal waters the less polluted ones (in terms of number of substances detected, DF and concentration levels). Although the removal efficiency (RE) in WWTP is adequate for most of compounds there are some substances, e.g. the pesticide fipronil or the pharmaceutical sulpiride, with low RE and whose levels remained high in the receiving medium, and even in points of the basin that were further away from the emission sources, endorsing the high M and P of them in the water cycle. In some cases, the concentration levels were higher than the predicted no-effect concentration (PNEC), posing a risk to aquatic organisms.

### 3.20.P PMT/vPvM substances: Assessment, Management and Regulation

#### 3.20.P-Th231 Exploring Data Availability and Variability for the Classification of Persistent and Mobile Substances

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Chemical hazard and risk assessments often use physical-chemical properties to categorize and identify chemicals of concerns. For persistence and mobility, we rely on simulated half-lives in different environmental compartments, organic carbon–water partition ratios ( $K_{OC}$ ), octanol–water partition ratios ( $K_{OW}$ ), octanol–water distribution ratios ( $D_{OW}$ ), and acid dissociation coefficients ( $pK_a$ ). The use of experimental data in these risk assessments is preferred but often not available. In a previous assessment, 41% of substances in the REACH registry and 36% of substances found in drinking water lacked reliable experimental and estimated property data to determine persistency and mobility. The OECD's eChemPortal allows for the extraction of experimental and estimated physical-chemical property data including those reported in the REACH dossiers. In this work we take a closer look at the availability of experimental data found using the OECD's eChemPortal property search function for the classification of persistent and mobile substances. We use a Bayesian analysis approach to include non-point estimate data,



such as maximum and minimum values, and interval ranges in our analysis. This approach will allow us to determine a consensus experimental value (and its uncertainty) based on all available data.

The project ZeroPM seeks to identify substance groups and substructures that are common in persistent and mobile substances. Thus, building a dataset of reliable physical-chemical property data for persistent and mobile categorization is critical. In this work, we take a closer look at the availability and variability of data in REACH dossiers for the classification of persistent and mobile substances. The data curated in this work will be used to identify substructures and functional groups commonly present in persistent and mobile substances.

### **3.20.P-Th232 "cleanventory": A Data Science Approach to Identify Persistent and Mobile Substances Regulated in Global Trade Markets**

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To enable data-driven decision making and to advance the ability to assess, manage, and regulate the use of persistent and mobile substances, a sound overview of the chemical diversity regulated within global trade markets is necessary. As part of the H2020 project ZeroPM (<https://zeropm.eu>), an open-source global chemical inventory – the "cleanventory" – is being developed to help identify, prioritize, and group persistent and mobile substances. A modern database infrastructure will facilitate wide-spread use of the database: it will be publicly available and will also provide programmatic access.

So far, 33 individual files from 18 inventories in twelve global trade markets are considered. These contain over 960,000 inventory entries with over 215,000 unique CAS Registry Numbers and over 360,000 unique chemical names. While this amount of information could be considered "big data", we put considerable efforts towards the quality of the data, therefore, also ensuring "good data".

Four API services are used to convert inventory identifiers to chemical structures: PubChem, CAS Common Chemistry, NCI/CADD Chemical Identifier Resolver, and ChemSpider. The workflow aggregates the structure information returned by the API services. To identify the "most probable" chemical structure for every inventory entry, a weighted consensus ranking approach was developed to assign each structure an identification score between 0 and 1.

The work on the "cleanventory" is ongoing and new data sources will be incorporated. These include important additions such as chemical substances regulated as pharmaceuticals, pesticides, and food-packaging materials. Workflows to identify "moieties of interest" for prioritization and grouping of persistent and mobile substances are being investigated. Future work will also explore the integration of fully defined chemical mixtures as well as polymers and UVCBs and explore the possibility of automatic integration of curated QSAR model predictions.

This high-quality "cleanventory" will enable effective prioritization and substance grouping strategies to help regulators, industry, and the water treatment sector achieve zero pollution of persistent and mobile substances.

### **3.20.P-Th233 Current status of the implementation of the new hazard classes PMT and vPvM into CLP and REACH regulation**

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The European Green Deal (EGD) sets an ambitious zero pollution vision for 2030. The overall objective is to reduce the pollution of air, water, and soil to levels that are not harmful to human health and the environment. In this context the European Commission published on October 14th, 2020, its Chemicals Strategy for Sustainability Towards a Toxic-Free Environment (CSS). The CSS is one important pillar of the Zero-Pollution Ambition for a Toxic Free Environment.

The European Union has one of the most comprehensive and ambitious legislations on chemicals globally. The Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; COM, 2006) and the Regulation on the Classification, Labelling and Packaging of hazardous substances (CLP) are the two horizontal legislations on chemicals in the European Union.

The European Commission aims to revise the REACH Regulation and to strengthen the CLP regulation as cornerstones of EU chemicals legislation. The "One substance, one assessment" process will ensure simplification and greater transparency of chemical hazard assessment. In 2021 the process started to amend the CLP Regulation to introduce new hazard classes on endocrine disruptors, PBTs/vPvBs and PMTs/vPvMs and apply them across all legislation.

On September 24th, 2021, the Commission proposed new hazard classes and criteria in the CLP Regulation to fully address environmental toxicity, persistency, mobility, and bioaccumulation. Initial discussion took place at ECHA's PBT expert group. The criteria for PBT/vPvB and PMT/vPvM have been discussed and subsequently commented on in writing by the MS CAs, industry, NGOs, and stakeholders. On September 20, 2022, the European Commission began a public consultation on a draft delegated regulation that would amend the CLP Regulation to add new hazard classes and their criteria e.g. for PMT and vPvM. The Final referral with CARACAL is planned for 29.11.2022 including the reflection of comments within the framework of WTO notification. Adoption is planned for 19.12.2022. The delegation act will then be directed to the European Parliament and Council and the final publication in the Official Journal is planned for March 2023.

We present the current status of the implementation of the PMT/vPvM criteria into CLP and REACH regulation since November 2022. We will also apply the new hazard classes PMT and vPvM to REACH registered substances detected in raw water and drinking water.

### **3.20.P-Th234 QSARs for abiotic degradation and biodegradation of chemicals in the environment**

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Persistent, mobile, and toxic (PMT) chemicals are perceived as an emerging threat to aquatic environments and drinking water quality. Due to their chemical properties and persistence these compounds can be transported from sources of release to groundwater or remote ecosystems and may also have relatively low removal efficiencies during water treatment. Therefore, it is essential to screen and prioritize potential PMT chemicals. Many factors govern the occurrence of a chemical in drinking water, including but not limited to persistence in soil and water (biodegradation, hydrolysis, and photolysis). By developing QSARs for environmental degradation this project will address uncertainty and data gaps in assessing water-soluble chemicals for persistence (P). We will develop databases by aggregating and curating measurements of biodegradation and hydrolysis from the literature. Based on the datasets we will develop new QSAR models for predicting biodegradation and hydrolysis predict half-lives. Most existing biodegradation QSARs are limited to qualitative predictions, or they have narrow applicability domains (AD) making quantitative predictions for specific chemical classes. We aim to expand the biodegradation database and developed QSAR models for quantitative or semi-quantitative estimates of biodegradation half-life with a wide AD. Hydrolysis QSARs in the literature have similar limitations, they are developed from rather limited datasets and focus on specific classes of compounds. To address this issue, we plan to collect and critically evaluate data on hydrolysis and build new comprehensive QSAR models for predicting hydrolysis the half-lives for organic chemicals.

### **3.20.P-Th235 Towards the Development of an Analytical Framework for the Determination of Persistent and Mobile Chemicals in Human Urine for Further Exploration of Human Exposure**

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There is a growing interest in the last decade on persistent, mobile, and toxic (PMT) / very persistent and very mobile (vPvM) chemicals (from herein abbreviated as PMs). Their high polarity (i.e., mobility in water) combined with persistence makes it difficult to remove them from the water cycle. Thus, they can constitute a potential harm for the aquatic environment and human health because they can break through natural and anthropogenic barriers into drinking water.

This high polar character also renders PMs hard to determine by conventional analytical methods, mostly relying on reversed-phase (RP) solid-phase extraction (SPE) combined to liquid chromatography-mass spectrometry (LC-MS). Several improvements have been introduced in the last years in the analysis of PMs in water by exploring other sample preparation and chromatographic approaches, such as hydrophilic interaction LC (HILIC), mixed-mode LC (MMLC) and supercritical fluid chromatography (SFC).

Despite those recent advances, there is so far no (comprehensive) knowledge on human exposure to PMs. This can be approached by biomonitoring, e.g., by urine analysis, if analytical methods are available. Therefore, in this work we aimed at moving forward toward the development of an analytical framework and build on former knowledge in water analysis, that could be then used for human urine analysis. To that end, we selected 38 different compounds with polar character (logD at pH 7 from -7.0 to 3.4) and different acid/base/permanent charge characteristics, to be used as model compounds. Then, the retention of these compounds was investigated on a total of 9 different columns from 4 different chromatographic modes (RPLC, MMLC, HILIC, and SFC) with pure standards and in matrix. Among the tested approaches, SFC with a Torus Diol column afforded the best retention and peak shape, while being less affected by matrix effects. On the other hand, we made a preliminary assessment of a generic SPE with large Oasis HLB (500 mg) cartridges as a preconcentration step at different pHs. This method allowed the satisfactory extraction (recoveries >80%) of up to 19 chemicals. Hence, further work is being directed towards a better coverage of PMs by improving this sample preparation step.

### **3.20.P-Th236 Evaluating the occurrence of and exposure to persistent and mobile chemicals using a comprehensive fate and exposure model PROTEX**

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Persistent, mobile, and toxic (PMT) chemicals are perceived as an emerging threat to aquatic environments and drinking water quality because they can penetrate through natural barriers and contaminate riverbank filtrate and groundwater. Different from “legacy” environmental pollutants, which are largely non-ionizable hydrophobic, PMT chemicals are polar or ionizable, with high hydrophilicity. Due to the low removal efficiency during wastewater and drinking water treatment, it is essential to identify potential PMT chemicals and quantify their environmental fate, human and ecological exposures, and potential risks.

Environmental fate and exposure models are cost-effective and user-friendly tools to achieve this goal, with one of the examples being the PROduction-To-EXposure (PROTEX) model. In this presentation, we report our efforts to upgrade and evaluate PROTEX to allow for (i) hydrological water balance and compartments (shallow and deep groundwater, representing riverbank filtrate and groundwater, respectively) relevant to chemical exposure through drinking water, (ii) partition behavior of ionizable chemicals in aqueous environments, and (iii) sorption of chemicals onto geosorbents other than natural organic matter, such as minerals and black carbon. For illustrative purposes, we apply the updated PROTEX model to predict concentrations of perfluorooctanoic acid (PFOA) in surface water, riverbank filtrate, and deep groundwater. The result shows that PFOA concentration in surface water is responsive to cessation of emissions, whereas it takes time for PFOA to be transported to and accumulated into shallow and deep groundwater. Such a time-lagging effect highlights the irreversibility of historical contamination of PMT chemicals. By using the updated PROTEX model, one can predict the time-dependent chemical concentration in different environmental compartments. In this case, regulatory efforts can leverage this information to understand the temporal accumulation of PMT chemicals and the irreversibility of historical contamination of PMT chemicals. This work

provides a holistic computational method to predict the environmental fate and human and ecological exposure for PMT chemicals.

### 3.20.P-Th237 Should we assess the P&M chemicals from a perspective of the “hazard” or “exposure”?

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Persistent and mobile (P&M) chemicals are considered emerging threats to the environment and drinking water because they can be transported over long distances, penetrate natural and artificial barriers, and resist removal by traditional water treatment procedures. Current chemical regulatory practices define P&M chemicals based on the presence of chemicals in drinking water and the potential for human exposure. However, when screening the myriad of commercial chemicals for mobility, most existing efforts utilize bright-line criteria based on chemicals' intrinsic “hazard” properties, such as biodegradation half-lives for P and organic-carbon-water partition coefficients for M. In this work, we compare and contrast the proposed intrinsic “hazard” criteria with chemicals' concentration in drinking water predicted by a fate and transport model, based on the data of 112,000+ discrete organic chemicals registered in different countries' chemical inventories. We evaluate the consistency of chemicals prioritized by “exposure” potential (chemicals with the highest concentrations in drinking water sources) and those by “hazard” assessment (chemicals with high P&M scores). We find that while chemicals with high P&M scores tend to possess a high potential to contaminate drinking water, P&M are not the only properties related to a chemical's potential to contaminate drinking water. An example is the low potential of volatile chemicals to contaminate drinking water, even if they have high scores in P&M. Thus, screening chemicals based on the P&M hazard indicators alone may, unfortunately, lead to “false positives” and “false negatives”. It is therefore inappropriate to evaluate P&M chemicals by using hazard indicators as a proxy. This work addresses the academic and regulatory need for a better understanding of the processes and properties related to drinking water contamination and for developing and evaluating scientifically defensible methods and criteria to identify and potentially regulate chemicals of concern for the safe and sustainable use of chemicals in commerce.

### 3.20.P-Th238 Tracking vPvM in Urban Waters of Barcelona

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On October 2022, the European Parliament proposed an amendment to Directive 2006/118/EC, which is the legislation governing groundwater (GW) quality and protection. In the Directive, pesticides were already regulated individually and together. The persistent mobile and toxic (PMTs) and very persistent and very mobile (vPvM) substances do not appear in these regulation lists. These kinds of compounds demonstrate a high frequency of detection in urban waters and specifically in groundwater bodies and they have the potential to cause adverse effects on human and environmental health. As polarity increases, common C18 reversed-phase chromatographic columns are less effective on retention. Thus, polar substances are less retained than hydrophobic compounds, in which other column chemistries are required such as normal phase (HILIC) or ion exchangers. However, the number of studies investigating the toxicity of urban water is still limited compared to studies on the presence of contaminants. Here, urban water (GW and runoff water) from Barcelona city has been analysed using an evaporative enrichment methodology to search for polar organic compounds of a wide range of uses from polar pharmaceuticals to industrial or tire wear substances.

The urban water samples were first filtered under vacuum at 0,7 µm employing glass fiber filters and next, 10 mL of water were enriched using vacuum-assisted evaporation (VAC) using a BÜCHI<sup>TM</sup> Syncore Plus®. The bath temperature was set to 55°C, 250 rpm orbital rotation, and a vacuum gradient to 20 mbar. Reconstitution was done using 100% H<sub>2</sub>O for the C18 column analysis and 95:5 (ACN:H<sub>2</sub>O) for HILIC or ion exchange column analysis. For chromatographic purposes, three different chromatographic column chemistries were selected to achieve a good separation (77 compounds with log D <0 analysed). Generally, compounds between log D = 0 to -2 were efficiently separated with the T3 column which for more polar compounds, the HILIC column demonstrated better performance. Finally, for some specific compounds, the very polar and ionic substances, the ion exchange column was selected.

PMTs and vPvM substances must be monitored to obtain high-quality drinking water. As they are challenging compounds, a simple evaporative enrichment showed the most trustworthy approach to preconcentrate these substances and up to three different column chemistries were employed.

### 3.20.P-Th239 Screening of very persistent and very mobile compounds in groundwater by LC-HRMS

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There is an urgent need to identify very persistent and very mobile (vPvM) substances present in the environment and their sources, to provide a basis for future monitoring, toxicity testing, and potential regulation. For this purpose, two analytical workflows were developed to screen vPvM in groundwater from the aquifer of the Besòs river delta in Barcelona (Spain) included in an in-house developed database.

The first one is based on enrichment by weak anion exchange solid phase extraction (WAX-SPE). In this case, 200mL of filtered water samples at pH 3 were extracted using Oasis<sup>®</sup> WAX. The chromatographic separation was performed using an Acquity<sup>TM</sup> Ultra High-Performance Liquid Chromatograph (UHPLC) system from Waters using a mixed-mode liquid chromatography (MMLC) Waters Atlantis Premier BEH C18 AX Column (2.1 mm id, 100 mm length, 2.5 µm particle size) from Waters, using as mobile phase 10 mM ammonium formate in water (A) and methanol (B). The chromatographic system was coupled to a HRMS equipped with a heated electrospray ionisation source HESI-II from Thermo Fisher Scientific working in negative mode.

The second approach consisted of a sample enrichment procedure based on the extraction and purification of 200 mL of filtered samples at pH 4 by weak cation exchange solid phase extraction (WCX-SPE) with Oasis®WCX from Waters followed by analysis using hydrophilic interaction liquid chromatography (HILIC) with a SeQuant®ZIC®-HILIC chromatographic column (2.1mm id, 100 mm length, 3.5µm particle size; Merck KGaA) using as mobile phases 10 mM ammonium acetate in water with 0.1% acetic acid, pH 4 (A) and acetonitrile with 0.1% acetic acid (B). The ionization source was a HESI-II operated in positive conditions. In both cases, mass spectrometry was fulfilled in a Thermo Scientific QExactive from Thermo Fisher Scientific with an Orbitrap analyser. The full scan data acquisition was in the range of 50-800 m/z at 70,000 full width at half maximum (FWHM) of resolution.

Most of the PMT/vPvM identified in the samples were PFAS with concentrations in groundwater ranging from 0.3 to 11500 ng/L. Short-chain compounds were in general predominant and PFPeA has been detected at higher concentrations. Other compounds that were detected in the samples were methyl 4-hydroxybenzoate, diphenil guanidine, sulfanilic acid, and venlafaxine.

### 3.20.P-Th240 Appearance in and Removal of PFAS From Tunnel Wash Water

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Road tunnels are constructions built under fjords or through mountains to allow traffic to move faster and safer from one place to another. For visibility and traffic safety, tunnels are cleaned regularly. After the wash, the tunnel wash water (TWW) remains as a highly polluted matrix, containing substances, such as trace elements (e.g., Cu, Zn, and Ni), polycyclic aromatic hydrocarbons (PAH), per- and polyfluorinated alkylated substances (PFAS), benzothiazoles (BTH), benzotriazoles (BTR), and their derivatives. Therefore, TWW needs to be treated before released into the municipal sewage system or to natural water bodies.

For two years, a treatment pilot has been operated and monitored to reduce the contaminant load in the TWW from a tunnel located close to Oslo, Norway. The Bjørnegård tunnel with two tubes is 2.3 km long and located in an urban area with an annual average daily traffic (AADT) count of approximately 17.000 vehicles per day in each tube. A sedimentation basin serves as a preliminary treatment step for the TWW for 35 days. This treatment step has been proven efficient for contaminants attached to particles, while the dissolved compounds are still present in the TWW. In this project the sedimented water was further treated with three different treatment steps: a system with two consecutive bag filters (5 and 1 µm mask openings), a ceramic membrane system with a pore size of 0.1 µm, and an adsorption column (220 cm high, 95 cm in diameter) filled with granulated activated carbon (GAC).

Grab samples of fresh and sedimented TWW, as well as before and after each secondary treatment step, were collected and analysed by LC-MS/MS to detect the presence and investigate the removal efficiencies of 22 PFAS in both, particulate and dissolved phase. Results show that several compounds from this class of pollutants are present in the TWW. In fresh TWW, the total sum of PFAS compounds ( $\Sigma_{22}$ PFAS) in the particulate phase was 0.14 µg/L, and 1.4 µg/L in the dissolved phase. A 96% removal efficiency was observed for particle bound  $\Sigma_{22}$ PFAS by sedimentation. For the dissolved  $\Sigma_{22}$ PFAS 76% removal efficiency was obtained by sedimentation and the secondary treatment step adsorption with GAC. The results of this study provide insight into the complex matrix that TWW represents and will assist road agencies in making decisions regarding the implementation of TWW treatment systems in new and existing road tunnels.

### 3.20.P-Th241 Occurrence of PMT/vPvM substances in groundwater of the Czech Republic

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PMT/vPvM substances were monitored in groundwater from 2021 to 2022. Artificial sweeteners saccharine and acesulfame, 1H-benzotriazole and 1,2,4-triazole as substances included in regular groundwater monitoring were analyzed in 1408 samples from 704 monitoring sites. The most frequently found substance was acesulfame (in 258 of 1408 samples), followed by 1,2,4-triazole (in 116 samples) and 1H-benzotriazole (in 93 samples), saccharine occurred just in 9 samples. Acesulfame occurred in concentration above 100 ng L<sup>-1</sup> in 163 samples, maximum concentration reached 2050 ng L<sup>-1</sup>. 1,2,4-triazole, 1H-benzotriazole and ccharine occurred in concentrations above 100 ng L<sup>-1</sup> in 7, 21, and 7 samples, reaching maximum concentration of 404, 12900, and 723 ng L<sup>-1</sup> respectively. Other 20 substances such as HEPES, Dimethyl 5-sulfoisophthalate sodium salt, Methacrylamido propyl trimethyl ammonium chloride, 3-Allyloxy-2-hydroxypropane sulfonate sodium salt, 4'-Aminoazobenzene-4-sulphonic acid, 4,4'-Diaminodiphenylmethane, 1-Ethyl-3-methylimidazolium ethyl sulfate, Dimethylamino propyl methacrylamide, N,N-Dimethylbenzylamine, 6-Methyl-1,3,5-triazine-2,4-diamine (Acetoguanamine), Benzyltrimethylammonium chloride, Naphthalene-1-sulfonate, Sodium ethylenesulphonate, Sodium methyl sulfate, 2-Methyl-2-propene-1-sulfonic acid sodium salt, 1-Adamantylamine, 2,4-Xylenesulfonic acid, 3,5-Di-tert-butylsalicylic acid, 2-Acrylamido-2-methyl-1-propanesulfonic acid sodium salt, and p-Toluenesulfonic acid were analyzed in 125 samples from 125 monitoring sites in 2021. Of those 9 compounds occurred in groundwater samples, p-Toluenesulfonic acid was found in all 125 samples, 3,5-Di-tert-butylsalicylic acid in 114 samples, Sodium methyl sulfate in 94 samples, 2,4-Xylenesulfonic acid in 92 samples, 1-Ethyl-3-methylimidazolium ethyl sulfate in 35 samples, Naphthalene-1-sulfonate in 31 samples, 1-Adamantylamine in 7 samples, 4,4'-Diaminodiphenylmethane in 4 samples, and Benzyltrimethylammonium chloride in 3 samples. Maximum concentrations of individual substances ranged between 44 ng L<sup>-1</sup> (Benzyltrimethylammonium chloride) and 8700 ng L<sup>-1</sup> (p-Toluenesulfonic acid). The other 11 substances were not found.

### 3.20.P-Th242 Nontarget Analysis of Water by Online Solid-Phase Extraction Coupled to Reversed Phase and Hydrophilic Interaction Liquid Chromatography with High-Resolution Mass Spectrometry Detection

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A wide variety of organic micropollutants have been reported in water resources, e.g., industrial and commercial chemicals, pharmaceuticals, per- and polyfluoroalkyl substances, and pesticides. Many other current-use chemicals have yet to be targeted in routine water analysis, representing unknown risks to ecosystems and human health. Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) based nontarget analysis has already evolved as a powerful approach to characterize micropollutants in waters. However, comprehensive chemical-space coverage is limited by the analytical method's performance for a wide range of analyte physical properties (e.g., polar, non-polar, acids, bases), including extraction recoveries, blank response, chromatographic separation, method sensitivity, choice of MS/MS (i.e. MS<sup>2</sup>) acquisition, and mass spectrometric resolution. To allow strategic prioritization of features for identification e.g., pollution patterns, or toxicological relevance, a high-throughput workflow collecting abundant data of satisfactory quality is essential. Increasing concern for persistent, mobile and/or toxic compounds, with their potential to accumulate in drinking water sources, have recently shifted the focus towards expanding the analytical coverage to include more polar micropollutants.

To tackle these challenges in nontarget analysis of water, we developed an online-solid phase extraction (SPE)-LC-HRMS workflow. The method requires minimal sample preparation, allowing high throughput and reduction of blank response. To expand the analytical coverage, a variety of column chemistries, i.e., reversed phase, HILIC, and mixed-mode, were assessed to improve online-SPE retention and chromatographic separation of 218 native standards, having predicted log P ranging from -4.6 to 11.2. MS<sup>2</sup> acquisition was optimized by comparing data dependent (top 10 precursors) and data-independent acquisition (5 precursor m/z windows) after data pre-processing and spectral deconvolution in MS-DIAL. Success rate of MS<sup>2</sup> library matching (MS-DIAL; GNPS), and in-silico structural predictions (SIRIUS; MS-Finder) for spiked standards in water matrices of varying complexity were evaluated for both MS<sup>2</sup> acquisition methods. The workflow was applied to groundwater, drinking water, surface water, storm water, and wastewater in Sweden to assess the spatial patterns of target analytes and nontarget features.

### 3.20.V PMT/vPvM substances: Assessment, Management and Regulation

#### 3.20.V-02 National-scale spatially-explicit health risks associated with persistent mobile and toxic (PMT) chemical exposure via drinking water

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Substances that are persistent, mobile, and toxic (PMT) have a greater propensity to breakthrough standard drinking water treatment processes because of their physico-chemical properties. Here, we present findings from a national-scale spatially-explicit risk assessment of drinking water sources in England and Wales for substances that meet proposed criteria for the hazard class PMT or vPvM. We screened a large number of substances and identified 22 chemicals with PMT/vPvM properties with a high likelihood of release to the environment. The principal exposure route for all substances was assumed to be via wastewater emission to surface waters followed by abstraction. A tiered assessment of exposure was performed with different tonnage tiers. In each case, we assumed consumption of wastewater effluent with and without water treatment. 3D-QSPR modelling was used for estimating the effectiveness for micropollutant removal with ozone- and chlorine-based treatment. Removal in WWTPs was calculated using SimpleTreat v4. The final tier assessment included dilution and riverine transport using spatially-referenced gridded model for the whole of Great Britain which was allowed exposure to be assessed at 296 locations where water is abstracted for drinking water supply. At each tier of the exposure assessment a risk characterisation ratio (RCR) was calculated, assuming that (i) exposure via drinking water only and (ii) only 20% of exposure to the chemical arises from the consumption of drinking water (with additional exposure from unknown sources:  $RCR_T$ ).

Most substances had a  $RCR < 1$  even in the very conservative lower tier screening assessments. Four of the 22 substances were identified as posing potential risks if no treatment were in place but only one substance had  $RCR_T > 1$  assuming conventional treatment. For 1,4-dioxane, spatially-referenced model predictions suggest that  $RCR_T$  was  $> 1$  for 37 abstraction locations (12.5%) under Q<sub>95</sub> (low flow) conditions, but only when a factor 5 safety factor was used accounting for additional exposure from unknown sources. Risks at these locations should be assessed further via site-specific investigations.

### 3.21.A Polymer Additives and Their Transformation Products as Chemicals of Emerging Concern: Environmental Emissions, Fate Processes, and Impacts

#### 3.21.A.T-01 Assessing the Effects of Acute and Pulsed Exposures of 6PPDq on Brook trout (*Salvelinus fontinalis*) Fingerlings and Fry

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Recent studies on the toxicity of stormwater runoff from urban areas have indicated that *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone) may be responsible for the mass mortality of Coho salmon (*Oncorhynchus kisutch*). Though Coho salmon are the most sensitive species found to date, phylogenetic similarity to Coho salmon is not a good predictor

of sensitivity to 6PPDq. The majority of toxicity tests performed so far have been 24-h static exposures to juvenile life stages of each species, the focus being on generating hazard data for a diverse array of fish species. However, due to the kinetics of stormwater runoff, the majority of environmental exposures are more likely to occur as brief pulses, and the effects of these exposures is not well understood. In this study we compared the effects of 24-h 6PPDq exposures to two life stages of brook trout, and the compared our 24-h LC50 values to the mortality data generated using pulsed exposures. Three different exposure designs were used for these studies; a standardized 24-h acute toxicity test for fry and fingerlings, a latent effects exposure where fish were exposed to the same time weighted average concentration (2 µg/L for 0.5-h, 1 µg/L for 1-h, 0.32 µg/L for 3-h, and 0.16 µg/L for 6-h), and repeat pulsed exposures where fish were exposed to 1 µg/L 6PPDq for 0.5-h, moved to clean water for 1-, 3-, or 6-h and then re-exposed for 0.5-h to the same concentration of 6PPDq. Brief exposures for very short durations resulted in higher mortality rates than longer exposure durations. For the repeated 0.5-h pulsed exposures, mortality rate was highest with the shortest gap between pulses (1-h), and there was no notable difference in the mortality rate observed with a 3- and 6-h gap. In our study, brief exposures at higher concentrations resulted in higher mortality rates than longer exposures at lower concentrations, which suggests there may be a threshold exposure concentration after which recovery is no longer possible. The results also highlight how quick 6PPDq uptake is occurring because 0.5-h exposures were long enough to cause 100% mortality, and the delayed onset of mortality that does not start to occur until ~2.5-h post exposure. This study highlights the importance of testing the toxicity of 6PPD-quinone on a range of different life stages of fishes and exposure regimes to better understand the impact of brief exposures that would likely occur in a stormwater runoff event.

### 3.21.A.T-02 Uptake and transformation kinetics of the tire rubber-derived contaminant 6-PPD and 6-PPD quinone in the zebrafish embryo (*Danio rerio*)

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As a widely used antioxidant in tire rubber, N-phenyl-N'-(1,3-dimethylbutyl)-p-phenylenediamine (6-PPD) and its toxic metabolite 6-PPD quinone (6-PPDQ) persistently enter surrounding aquatic environments via roadway runoff. 6-PPD and 6-PPDQ showed toxicity toward various aquatic organisms and different fish species showed differential susceptibility to them. In contrast to other fish species (e.g., coho salmon), zebrafish are relatively tolerant to 6-PPD and 6-PPDQ. However, it is unclear whether the tolerance is attributed to the biotransformation of 6-PPD and 6-PPDQ. We hypothesized that 6-PPD and 6-PPDQ undergo biotransformation, leading to their detoxification in zebrafish. To validate our hypothesis, we investigated the uptake and biotransformation of 6-PPD and 6-PPDQ in zebrafish embryos. Both 6-PPD and 6-PPDQ were quickly taken up by the zebrafish embryo and internal concentration reached a maximum after a 6 h-exposure and then decreased. Moreover, we observed extensive phase II conjugations of 6-PPD, 6-PPDQ, and their oxidated transformation products. Predominant conjugates include glutathione, acetylcysteine, and glucuronide, which may contribute to zebrafish's tolerance to 6-PPDQ. Further work is needed to compare the biotransformation in other 6-PPD-sensitive species and determine the contribution of biotransformation to species-specific toxicity.

### 3.21.A.T-03 Toxicity of 6PPD-quinone to Early-life Stage Rainbow Trout

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Recently, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), a rubber- tire derivative that leaches from tire wear particles into roadway runoff, has been identified as a driver of urban runoff mortality syndrome in coho salmon. Sensitivity to this toxicant is highly variable across fish species with potential for ecologically significant effects on relevant species is significant. Rainbow trout (*Oncorhynchus mykiss*) represent a model species which are known to be sensitive to 6PPD-quinone. Given that early life stages of fishes are typically more sensitive than adult, this study aimed to evaluate the toxicity of 6PPD-quinone to early-life stage rainbow trout. Eyed embryos were obtained from Lyndon Hatcheries and alevins were exposed from hatch to time-weighted average (TWA) concentrations of 6PPD-quinone of 2.35, 1.30, 0.44, 0.20, 0.10, and 0.06 µg/L, as well as a water and solvent (0.01% dimethyl sulfoxide) control in quintuplicates. Fish were kept at 14±0.5°C, on a 16:8-h light: dark period under semi-static conditions, with a 70% water change each day. Alevins were exposed for 28 days, after which they were euthanized in buffered 200 mg/L tricaine mesylate (MS-222), weighed and measured, and samples taken for histological and biochemical analysis. Chronic exposure to 6PPD-quinone resulted in significant mortality at concentrations at and greater than 0.44 µg/L. The 28-day LC50 was 0.56 µg/L and defects occurred in the three highest concentrations, including spinal curvature, yolk sac edema, and notably, pooling blood in the caudal fin. An acute 96-h exposure study was also performed, using five-week post-hatch rainbow trout fry. The 96-h LC50 for five-week post-hatch fry was 0.47 µg/L. From this study, we can conclude that early-life stage rainbow trout alevins are more sensitive than sub-adults, with five-week post-hatch fry exhibiting the greatest sensitivity to acute exposure with 6PPD-quinone.

### 3.21.A.T-04 Acute Cardiometabolic Responses of Juvenile Salmonids Exposed to 6PPD-Quinone

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely

used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, this compound has been reported to cause acute lethality at approximately  $\leq 1$  ug/L in a variety of salmonid species including coho salmon, rainbow trout, and brook trout. However, additional studies have shown other salmonid species such as arctic char and bull trout to be insensitive, even at significantly greater concentrations (20 ug/L). Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. We investigated the acute cardiorespiratory effects of 6PPD-quinone to two salmonids of differing sensitivity: a sensitive species, rainbow trout, and a tolerant species, Arctic char. Fish were exposed to 1 ug/L or 10 ug/L 6PPD-quinone in respirometry chambers for 48 h to assess temporal changes in resting oxygen consumption compared to unexposed controls. Following exposure, cardiac ultrasound was used to characterize cardiac function by analyzing changes in ejection velocity, stroke volume, ventricular and atrial contractile rates, and cardiac output. Furthermore, electrocardiography was used to determine changes in the heart's electrical activity, and blood gas analyses was used to analyze changes in 19 parameters of the blood. Preliminary data show that 6PPD-quinone exposure does not significantly impact oxygen consumption rates in either species. In contrast, exposure appears to cause a significant increase in passive ventricular filling in both Arctic char and rainbow trout. In addition, in just rainbow trout, a decrease in end systolic volume and increase in atrial and ventricular contractile rates were observed, providing evidence of sympathetic stimulation. Cardiorespiratory symptoms observed in rainbow trout exposure might partly be driven by a significant increase in methemoglobin, resulting in an impaired ability to oxygenate the tissues. This is the first study to analyze the toxicity of 6PPD-quinone to the cardiorespiratory system of fishes of commercial, cultural, and ecological importance at environmentally relevant concentrations and provides information invaluable to a better understanding of the mechanism of 6PPD-quinone toxicity.

### 3.21.A.T-05 Prioritization of consumer plastics for further testing based on artificial weathering combined with bioanalytical and chemical screening

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The ever-increasing plastic pollution poses a threat to the aquatic environment. While the formation of microplastic particles has been extensively investigated, leaching of plastic additives only recently became a major research interest. They account for up to 70% of the polymer and are not chemically bound to the polymer backbone. Plastic additives can leach into the environment where they may be transformed and bioaccumulate. Toxic effects have been reported for plastic leachates, which may be driven by additives from different classes, such as metals, antioxidants, flame retardants, or UV-stabilizers. The project P-LEACH ([www.ufz.de/p-leach](http://www.ufz.de/p-leach)) systematically addresses the investigation of the leaching of additives from selected consumer products combined with chemical analysis and biological effect screening.

Seven polymer materials from different consumer applications were purchased to investigate the potential impacts of related leachates to the environment. Subsequently the materials were weathered in an artificial weathering wheel. After weathering, the toxicity of the leachates was evaluated by exposing synchronized *S. vacuolatus*. The leachates were screened in a non-targeted approach using a reversed phase HPLC–HRMS-system. For comprehensive analysis, leachates and acid digests of the raw, UV-treated and dark control materials were also screened for 55 metals and metalloids by ICP-MS/MS.

Our study indicates the relevance of leachates in plastic consumer products for phytotoxicity. The compact study design enabled us to rank plastic materials in their potential risk toward freshwater algae. The non-target screening workflow used will help identify additives contributing to the observed toxicity and provide insight into their release characteristics regarding heavy metals and organic constituents. In future experiments it will be possible to compare custom-made polymer materials with known patterns and mass fractions of additives to commercially manufactured consumer products.

### 3.21.B Polymer Additives and Their Transformation Products as Chemicals of Emerging Concern: Environmental Emissions, Fate Processes, and Impacts

#### 3.21.B.T-01 Improved Kinetic and Mechanistic Understanding of Atmospheric Chemical Transformations of Polymer Additives Through Laboratory Studies

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Vehicle tires are a complex mixture of chemicals that are generally added to the rubber to increase their durability and stability. Tire wear related chemicals are expected to be emitted to the atmosphere through mechanical processes, particularly in a near road environment as PM<sub>2.5</sub> and PM<sub>10</sub>. Such tire wear particles (TWP), contain a wide array of organic contaminants such as microplastics, benzothioles, polycyclic aromatic hydrocarbons, and amines, some of which have been implicated as having toxic environmental impacts. While there is significant literature on the fate and toxicity of these particles (and chemicals therein) in aquatic environments and surface waters, there is limited knowledge about their fate and toxicity as a result of exposure to the atmosphere. An important class of tire wear chemicals are the substituted phenylamines, which are frequently used as anti-oxidants during the manufacturing of tires, and contain multiple phenyl groups and secondary amine functionality. Recent studies have indicated the presence of such a molecules (6-PPD in particular and its oxidation products) in surface waters impacted by highway and road runoff. However, 6-PPD and the subsequent detected quinone product (6-PPDQ) represents a single parent – transformation product pair, out of the dozens of antioxidants used, and potentially hundreds of transformation products formed.

In addition, the transformation chemistry expected to occur in the atmosphere (especially in a near road environment) will be rather different than what is likely to occur within surface water, and yet little is known about the atmospheric chemical reactions and fate of these antioxidants. In our study, a series of laboratory experiments are performed using atmospherically relevant conditions and real-time instrumentation to investigate the kinetics associated with heterogeneous oxidation of 6-PPD and DPPD in air and to derive atmospherically relevant chemical transformation mechanisms leading to a range of previously unidentified oxidation products. The results indicate that both OH radical and ozone reactions are important oxidation pathways, with >40 different products formed, including both aromatic ring retaining and ring opening products of diverse functionality. Of particular importance is the formation of highly toxic nitrosamines and nitramines which form in the presence of NO<sub>x</sub>. The atmospheric oxidation of tire wear antioxidants found to be rapid and possibly as important as other aquatic oxidation mechanisms, leading to different and potentially toxic species. The atmospheric oxidation products observed warrant further scrutiny with respect to their inherent toxicity and their prevalence in atmospheric and/or aquatic samples from the ambient environment.

### 3.21.B.T-02 Plant Uptake and Metabolism of Tire-derived Compounds

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Tire wear particles are expected to reach agricultural fields via biosolids application, atmospheric deposition, or irrigation with recycled water. In the field, tire wear particles will release a suite of organic additives in the root zone of plants. These compounds are then available for uptake by edible plants, which could be a route for human exposure to tire-derived compounds, as has been previously shown for pharmaceuticals and personal care products. In this study, we exposed lettuce plants to tire-derived compounds and tire particles and monitored the concentration of five common tire-derived compounds: hexamethoxymethylmelamine (HMMM), benzothiazole (BTZ), 1,3-diphenylguanidine (DPG), N-phenyl-N'-(1,3-dimethylbutyl)-p-phenylenediamine (6PPD), and 6PPD-quinone (6PPD-q) in lettuce roots and leaves. We found that all five compounds were taken up and translocated to lettuce leaves. Translocation was compound dependent and was hindered by differential affinity of compounds to lettuce root tissues. This affinity was driven by varying logK<sub>ow</sub> and charge of the compounds. All five tire-derived compounds were metabolized in lettuce plants, and we used untargeted high resolution mass spectrometry to identify the transformation products. We found that the five compounds were transformed via various metabolic processes within the plants. We identified novel transformation products of several compounds, including HMMM and 6PPD-q. We also found that for all compounds, except 6PPD, the transformation products formed were highly stable in the lettuce leaves. In our second set of experiments, when we exposed lettuce to tire particles, the long-term leaching of tire-derived compounds from the particles provided a resupply of these compounds and was able to overcompensate for the rate of metabolism in plants, leading to continuously increasing concentrations of the tire-derived compounds in the lettuce leaves over the duration of the experiments. Altogether, our findings indicate that if plants are exposed to tire wear particles during growth, the compounds contained in the tire wear particles may be taken up by plants, and these compounds and their transformation products may be present in the edible parts of the plants up through the time of consumption. This work highlights an important potential route of human exposure to tire-derived compounds.

### 3.21.B.T-03 Products and Reaction Pathways of the Multiphase OH Radical Oxidation of 2-Ethylhexyl Phthalate in Pure Films and Indoor Dust

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Today, many people spend most of their time indoors where they have the potential to be exposed to emissions of chemical contaminants from consumer products. While many of these primary emission sources are relatively well-understood, all indoor contaminants have the potential to react over time, whether in the gas phase or the condensed phase where most hydrophobic organic contaminants are likely to be found. One of the most universal oxidizing agents is the OH radical, which is present at low but relevant concentrations indoors and which has been shown to heterogeneously react with organic compounds over timescales of weeks to months. However, the products of multiphase OH radical oxidation of organic contaminants are not well-understood. We have shown that thin films of di(2-ethylhexyl) phthalate (DEHP), a common contaminant with significant human toxicity, heterogeneously react with OH radicals to produce more than 10 unique condensed-phase products identified using liquid chromatography coupled to a high-resolution mass spectrometer (LC-HRMS). Many of the observed products have multiple structural isomers and some have been shown to be more potent toxicants than the parent compound. These products exist in indoor dust, and we observe additional product formation following further OH oxidation of a standard reference material dust sample containing high concentrations of DEHP. Product semi-quantification is accomplished using a nonlinear regressor between predicted chemical descriptors and normalized MS signal response trained on a standard curve of a mixture of compounds with similar structural features, and tentative structural assignments are made based on a weight-of-evidence approach using exact mass analysis, retention time calibration, and existing knowledge of OH radical reaction mechanisms. This study is the first to characterize the complex mixture of transformation products resulting from multiphase OH oxidation of indoor contaminants, expanding the range of anthropogenically-derived organic compounds humans are likely exposed to indoors. Little is known about the toxicological properties of most of the transformation products observed, indicating that future work examining the effects of exposure to indoor heterogeneous transformation products on human health is critical to understanding the health effects of human xenobiotic exposure in the indoor environment.

### 3.21.B.T-04 Formation potential and in-vitro toxicity of chlorinated disinfection by-products of the polymer additive 1,3-diphenylguanidine (DPG)

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1,3-diphenylguanidine (DPG) is a commonly used rubber and polymer additive, that has been found to be one of the main leachate products of tire wear particles and from HDPE pipes, with recent estimates putting the yearly usage of DPG at up to 10,000 metric tons in Europe alone. Its introduction to aquatic environments and potentially water supplies lead to further questions regarding the effects of disinfection by-products potentially formed. We present a comprehensive study of the potential toxic and genotoxic effects of chlorinated by-products of DPG; as well as their potential formation under conditions like those found during water treatment disinfection processes and within water distribution networks. Using different bioassay approaches and NGS RNA-sequencing, we show that some of the chlorinated by-products of DPG exert significant toxicity. DPG and its chlorinated by-products also can alter cell bioenergetic processes, affecting cellular basal respiration rates and ATP production, moreover, DPG and two of its chlorination products, 1,3-bis-(4-chlorophenyl)guanidine and 1-(4-chlorophenyl)-3-(2,4-dichlorophenyl)guanidine, have an impact on mitochondrial proton leak, which is an indicator of mitochondria damage. Evidence of genotoxic effects in the form of DNA double strand breaks (DSBs) was suggested by RNA-sequencing results and further validated by an increased expression of genes associated with DNA damage response (DDR), specifically the canonical non-homologous end joining (c-NHEJ) pathway. Immunofluorescence analysis of phosphorylated histone H2AX, another DSB biomarker, also confirmed the potential genotoxic effects observed for the chlorinated products. In addition, chlorination of DPG leads to the formation of different chlorinated products, with the 5 analyzed compounds in this work representing up to 42% of formed products. These findings indicate that DPG reaction with free chlorine doses commonly applied during drinking water treatment or in water distribution networks (0.2–0.5 mg/L) can lead to the formation of toxic and genotoxic chlorinated products. While the potentially genotoxic 1,3-bis-(4-chlorophenyl)guanidine product is formed at lower concentrations than less toxic products (1,3-bis-(2-chlorophenyl)guanidine and 1-(2,4-dichlorophenyl)-3-phenylguanidine) it could still pose a threat to human and environmental health.

### 3.21.B.T-05 Are Wastewater Treatment Plants an Effective Barrier Against Tire Leachables?-Biotransformation and Screening Experiment

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Tire and road wear particle act as source for a variety of organic and inorganic contaminants that can be released into the aquatic environment. Yet, only few so-called tire leachables are known as systematic studies of tire leachables and their environmental fate are scarce. A potential entrance and distribution pathway of tire leachables into the aquatic environment are combined sewer systems with wastewater treatment plants (WWTPs). To investigate the relevance of WWTPs as entrance pathway for tire leachables their biotransformation was investigated with tire leachates of new and used tires and with eleven standards of known tire leachables (N-tert-butyl-2-benzothiazolesulfenamide, N-cyclohexyl-2-benzothiazolesulfenamide, dibenzylamine, 1,3-dicyclohexylurea, 1,3-diphenylguanidine, 1,3-diphenylurea, 2-hydroxybenzothiazole, 4-hydroxydiphenylamine, 2-mercaptobenzothiazole, 6-PPD-quinone, tributylamine). The biotransformation experiment was carried out over 28 days in the dark and under aerobic conditions. Sample aliquots were taken regularly and analysed with RPLC-ESI-QTOF MS. Eventually, WWTP samples were retrospective evaluated to validate the results generated by the laboratory experiments.

The majority of investigated tire leachables was found to be primary transformed and 63 transformation products (TPs) were tentatively identified. For each TP the precursor assignment and structure elucidation were attempted but has proven problematic due to the complexity of the tire leachates. For the TPs of the standards the assignment was more feasible and noteworthy are 13 identified TPs for 2-mercaptobenzothiazole and two identified 6-PPD-quinone TPs. For most assigned TPs, structures could be identified, which corresponded mainly to the methylated, hydroxylated, or oxidised precursors.

The comparison of the laboratory experiment with full scale WWTPs showed a general compliance of the results. However, a transformation of up to 100 % for some of tire leachables as seen in the laboratory experiment could not be observed in the sampled WWTPs but only a partial decrease. This is attributed to the lag phase of several tire leachables of up to 11 days. Hence, WWTPs represent a possible entry pathway and contribute to the distribution of tire leachables and their TPs in the aquatic environment. Based on these results extensive monitoring studies are required to allow a risk assessment of the different tire leachables and their newly identified TPs.

### 3.21.C Polymer Additives and Their Transformation Products as Chemicals of Emerging Concern: Environmental Emissions, Fate Processes, and Impacts

#### 3.21.C.T-01 Tire-derived Compounds in the Atmospheric Environment

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Investigations of environmental tire contamination have mostly been limited to aquatic systems, and there are few studies pertaining to gaseous and particulate-sorbed contaminants that originate from tire wear in the atmospheric environment. In this study, to acquire an understanding of the current state of atmospheric tire contamination, a variety of air samples from across the globe were monitored for tire pollution. Using ultra-pressure liquid chromatography coupled with high-resolution mass spectrometry, archived passive air sampler extracts, air filter samples, and organic film samples were analyzed using both targeted

and non-targeted methods. First, to better understand the global ubiquity of this form of air pollution, samples from major cities across the globe, under the Global Atmospheric Passive Sampling (GAPS) Network (GAPS-Megacities) initiative, were analyzed and screened for tire-compounds of emerging concern. Secondly, to study the contamination trend of tire-derived chemicals on a regional scale, temporally-resolved samples from various source sectors, including roadside, urban, industrial, and residential areas in the Greater Toronto Area, ON, Canada were also investigated. Air sample filters deployed near high-traffic sites in Toronto were also screened to determine environmental levels of emerging transformation products of concern of common tire chemicals. Finally, to further investigate air exposures of tire-derived chemicals in urban settings, organic film samples were acquired by wiping impervious surfaces in areas exposed to high rubber tire use (Montreal, QC, Canada). For comparison, organic film samples were also acquired from locations that are more isolated from this pollution source (Montreal, QC and Toronto, ON, Canada). This study presents some of the first data for airborne concentrations of chemicals associated with tire-wear that can be used for modeling and exposure assessments. Due to the presence of many tire-derived contaminants in urban air across the globe, and the current lack of toxicity data for these chemicals, this study highlights the need for human health considerations pertaining to tire-derived chemicals.

### 3.21.C.T-02 Spatial and temporal trends for regulated plasticizers and their emerging substitutes in biota and non-biota samples from German rivers

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The objective of the new European Chemicals Strategy is effective protection of citizens and the environment by sustainable chemicals management. Restrictions of hazardous substances in manufacture, use or placing on the market are already instruments of risk management. However, are existing measures sufficiently sustainable? Plasticizers are high production volume chemicals used as main additives in the manufacture of flexible PVC. Due to their endocrine effects, the former dominant plasticizer Di(2-ethylhexyl) phthalate (DEHP) and some short-chain phthalates were banned in the EU. As a result, their concentrations have declined significantly in suspended particulate matter (SPM) from freshwater environments and DEHP is now mainly replaced by Di(2-propylheptyl) phthalate (DPHP) and by non-phthalates. DPHP has a high structural similarity to DEHP and its further marketing is under regulatory scrutiny as it has been flagged as a potential endocrine disruptor (REACH CoRAP). Until now, data are missing on the presence of DPHP and other substitutes in freshwater biota.

We analyzed zebra mussels *Dreissena polymorpha* and quagga mussels *Dreissena bugensis* for the presence of 16 plasticizers, i.e., 12 phthalates and 4 non-phthalates. The samples were retrieved from the German Environmental Specimen Bank (ESB) between the early 2000s and 2020 and covered sampling sites from major rivers, including Rhine, Elbe, and Danube.

In agreement with the SPM investigations, current mussel samples from all ten river sites in Germany showed DEHP substitutes. Our results indicate a fast appearance of DPHP as the main replacement plasticizer and of non-phthalates in both freshwater biota and SPM. Today, the high molecular weight phthalates Diisononyl phthalate (DINP) and Diisodecyl phthalate (DIDP) were the most abundant plasticizers detected in mussel samples from the rivers Rhine, Saar, and Danube. These results confirmed our spatial and temporal study in SPM. However, contrary to the SPM study and despite the strict EU regulations, DEHP has not dropped in general and was the dominant plasticizer in current mussel samples at the most sites from river Elbe (up to 80% of the total plasticizer load). Comparing both mussel species, *D. bugensis* showed higher concentrations of DEHP and its substitutes than *D. polymorpha*. These findings clearly highlight the need for better linking of environmental monitoring data with chemical legislation.

### 3.21.C.T-03 New Alternative Plasticizers in Foodstuffs: Occurrence, Migration from Packaging and Exposure Through Diet

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Plasticizers and flame retardants represent 47% of total additives in plastic products. Nowadays, phthalates have been replaced by other substances apparently less harmful, such as citric acid esters, and adipates. Acetyl tributyl citrate (ATBC) is a plasticizer commonly employed as phthalate substitute in food-contact materials, cosmetics, toys, or medical devices. Some studies described cytotoxicity and gene activation effects on organisms exposed to this compound. Di(2-ethylhexyl) adipate (DEHA) is frequently used in flexible PVC films. Both compounds are easily migrated from food packaging materials, which is another route of exposure.

The main objective of our study is to assess the exposure to alternative plasticizers through foodstuffs ingestion. Eighty-eight samples from different groups of foodstuffs (baby foods, cereals and legumes, chocolate, condiments, dairy products, eggs, fish, fruit and vegetables, meat, and oils) were selected. Four alternative plasticizers (ATBC, DEHA, diisononyl adipate (DINA) and 1,2-cyclohexanedicarboxylic acid 1,2-diisononyl ester (DINCH)), nine phthalates and twenty organophosphate esters were included in the instrumental LC-MS/MS method. In addition, migration tests were carried out with different packed food (vegetables, purée, potato omelette, and meat), to determine contamination of chemicals from plastic food contact materials (FCMs) used for microwave and conventional oven heating (roasting bags, microwavable plastic trays and microwave oven bags).

Results showed the presence of new alternative plasticizers in human diet, which is an outcome of plastic pollution. ATBC was the compound with the highest concentration in sweeteners (14.2 mg/g wet wt), followed by DEHA in serrano ham (10.6 mg/g wet wt), ATBC in fish baby food (3.38 mg/g wet wt) and meat baby food (3.35 mg/g wet wt), and DEHA in semi-cured cheese (3.30 mg/g wet wt) and fresh salmon (2.95 mg/g wet wt). Infants and toddlers were the most vulnerable population groups to plastic additives exposure. New plasticizers had higher presence than traditional phthalates. Moreover, migration from FCMs of

ATBC phthalate substitute increased with cooking. Hence, it is required to carry out more toxicity assessment studies of these new additives in humans, and the environmental consequences of their use. Overall, detection of chemicals associated to plastic pollution in foodstuffs may contribute to develop a restrictive legislation in the use of plastics for food packaging.

### 3.21.C.T-04 Chemicals Present in Plastics Pose a Risk to Health, Environment, and the Transition to a Circular Economy

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Plastics are widely used materials employed in various industrial sectors. They can contain various chemical substances, including residual monomers, additives, processing aids, and non-intentionally added substances (NIAS), which can be released and negatively impact human and environmental health and the circular economy. Information on the presence and concentration of individual substances in specific plastics products is scarce and scattered. In this study, we aim to fill this gap by systematically reviewing recent measurement studies and conducting a case-study on polyvinyl chloride (PVC) building materials in Switzerland. More than 3000 substances have been detected in different products in recent literature, however, approximately 75% of these substances were only found in non-targeted workflows, targeted workflows, and quantification efforts focused on few typically well-regulated substances (e.g., metals, brominated flame retardants, phthalates). Our PVC building materials' case-study also focused on these well-regulated substances (i.e., metals, phthalates); surprisingly, many of the new samples still contained toxic heavy metals (mainly lead), restricted phthalates (mainly bis(2-ethylhexyl) phthalate [DEHP]) or alternative phthalates (mainly diisononyl phthalate [DiNP]). The increased presence of lead and DEHP in products made from recycled material points to phased-out hazardous substances being retained in the economy by recycling. Overall, non-targeted studies show that many more substances than regularly measured in targeted analysis may be present in plastic products. At the same time, even when focusing solely on well-known and regulated substances, many new products on the market still contain them and thus can pose a risk to human health, the environment, and the transition to a circular economy, as shown in our case study of building materials. Given the immense workload needed for large-scale measurement campaigns, even the best efforts by the monitoring community will not be sufficient to comprehensively understand the presence and levels of chemicals present in specific plastic products to guide the safe use and recycling of such products. Therefore, concerted action is urgently needed to ensure transparency of chemicals in plastics.

### 3.21.C.T-05 Toxicological and Chemical Evaluation of Leaching Substances from Conventional and Bioplastics - A Holistic Approach

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Global production of plastics is increasing yearly. This trend is of environmental concern because hazardous chemicals can be released from plastic products. Polymers are not considered to be toxically hazardous because they are inert and their transport through biological membranes is limited. The release of additives, non-polymerized monomers, by-products and the formation of destruction and transformation products is of high environmental concern because these substances can provoke toxic effects. The aim of this study was to investigate toxicity of conventional and biodegradable plastics by acute and chronic testing with *Daphnia magna* in addition to chemical analyzation of toxic substances. Two fishing lures based on soft polyvinyl chloride (PVC) and four biodegradable plastic compounds were ecotoxicologically investigated.

Leachates of fishing lures and bioplastic compounds were prepared with ISO water. The acute and chronic test with *D. magna* was performed according to OECD Guidelines 202 and 211, respectively. The test was conducted using dilutions from the leachate stock solutions to determine EC50 values for comparison purposes. The stock solutions were extracted by liquid-liquid extraction and analyzed by GC-MS. Hydrophobic organic substances such as PAHs, bisphenol A, and phthalates were quantitatively determined. Qualitative screening for non-target substances was also performed.

Leaching of contaminants from plastics has shown strong effects in acute tests of fishing lures and chronic tests of bioplastics. Chemical analysis indicated correlations between the leached chemicals and the toxic effects of the plastics. A holistic approach that includes both ecotoxicology and chemical analysis is needed to understand how and why plastic compounds provoke effects. The identification of toxic substances is necessary mainly because additives do not have to be labeled and bioplastics can be as toxic as conventional plastics.

### 3.21.P Polymer Additives and Their Transformation Products as Chemicals of Emerging Concern: Environmental Emissions, Fate Processes, and Impacts

#### 3.21.P-Tu314 Acute Toxicity of 6PPD-quinone Across Fishes of Commercial, Cultural, and Ecological Importance

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), a transformation product of the rubber tire

antioxidant 6PPD, has been shown to be acutely toxic to coho salmon with a median lethal concentration (LC50) of 0.095 µg/L. Interestingly, subsequent studies have failed to confirm comparable sensitivity in a few other select fish species including chum salmon, medaka and zebrafish. This study aimed to assess the acute toxicity of 6PPD-quinone to seven fishes of commercial, cultural, and ecological importance in North America, rainbow trout, brook trout, westslope cutthroat trout, brown trout, bull trout, Arctic char, and white sturgeon. Furthermore, in vitro experiments with rainbow trout liver and gill cells were conducted to characterize the potential of 6PPD-quinone to affect mitochondrial respiration, which has been hypothesized as a possible mechanism of toxic action. This study revealed dramatic differences in the acute toxicity of the rubber-tire transformation product 6PPD-quinone across the tested fishes that may have significant implications for ecological risk assessment of urban runoff events. No mortality occurred for Arctic char, brown trout, bull trout, westslope cutthroat trout, and white sturgeon, while brook and rainbow trout were sensitive to 6PPD-quinone exposure with LC50 values of 0.59 and 1.00 mg/L, respectively. Even species from the same genus showed vastly different responses (*O. mykiss* vs *O. keta*; *S. alpinus* vs *S. fontinalis*). Initial experiments with primary cultures of rainbow trout gill cells suggest that the mechanism of toxicity may be related to uncoupling the mitochondrial electron transport chain. Further research is needed to characterize the mechanism driving the dramatic differences in sensitivity of fishes to 6PPD-quinone.

### 3.21.P-Tu315 Mutagenicity In Vitro and In Vivo of 6PPD Quinone, a Rubber Tire Oxidant By-Product

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD quinone) is a transformation product of the tire rubber antioxidant 6PPD. 6PPD quinone reaches the aquatic environment by stormwater and urban runoff and was recently associated with high mortality of salmon. There is still limited information about the toxicity of 6PPD quinone in different aquatic species; but it is clear that among the species analyzed they range from extreme sensitive to no effect. The objective of this study was to determine the acute toxicity of 6PPD quinone in the marine amphipod *Parhyale hawaiiensis* and verify its mutagenicity in vitro (Salmonella/microsome assay - Microplate agar) and in vivo (micronuclei in *P. hawaiiensis*). 6PPD quinone was dissolved in DMSO at the limit of solubility (5000mg/L). Acute toxicity of *P. hawaiiensis* neonates (<7 days old) was evaluated in 5 concentrations ranging from 31.25 to 500µg/L. For the in vitro mutagenicity assessment, 6PPD quinone was tested in concentration-response experiments from 0.125 to 100mg/L. Five strains of Salmonella/microsome assay (YG1041, TA100, TA98, TA102, and TA1535) were used with and without metabolic activation (rat liver S9, 5%). For the in vivo assessment, *P. hawaiiensis* adults (<8 months old) were exposed to two concentrations of 6PPD quinone (250 and 500µg/L) and the hemolymph was collected to evaluate the micronuclei frequency in the hemocytes. No acute toxicity was observed in *P. hawaiiensis*. 6PPD quinone showed mutagenic effect only in TA100 in the presence of S9, indicating that it requires metabolic activation. With 5% S9, a weak increase in the number of revertants/well was observed starting from 6.25mg/L in the two independent experiments, and with 10% S9 the increase started at 12.5mg/L. An increase in the micronuclei frequency in the hemocytes of *P. hawaiiensis* was observed after exposure to both concentrations of 6PPD quinone compared to the negative control. We conclude that 6PPD quinone was weakly mutagenic in the in vitro test and induced micronuclei in *P. hawaiiensis*. This mutagenic activity could be a concern for the less sensitive species. Because DNA damage in the reproductive cells can be easier correlated to populational level adverse effects, our next step will be to verify if this compound is able to damage DNA (comet assay) in the sperm cells of *P. hawaiiensis*.

### 3.21.P-Tu316 Potential Toxicokinetic Mechanism for 6PPD-Quinone Toxicity: Cross-Species Biotransformation and Metabolite Identification

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N-(1,3-Dimethylbutyl)-N'-Phenyl-P-Phenylenediamine-Quinone (6PPD-Q) is an abiotic transformation product of N-(1,3-Dimethylbutyl)-N'-Phenyl-P-Phenylenediamine (6PPD) found in rubber tires. Tire wear particles accumulate on roads and are washed into waterbodies via runoff resulting in maximum 6PPD-Q concentrations of 1.4 µg/L along the South Saskatchewan River. This is concerning because acute toxicity (< 96 h) to select salmonids occurs at 0.095 µg/L. There are considerable differences to interspecific responses without explanation. However, the proposed toxicity mechanism appears well-conserved across species. This study utilized ex situ isolated liver perfusion assays, in conjunction with in vivo bile samples to quantify the extent of 6PPD-Q biotransformation and qualitatively determine respective metabolites among fish species.

In vivo exposure of coho salmon, brook trout, rainbow trout, Atlantic salmon, westslope cutthroat trout, white sturgeon, and Arctic char yielded bile samples for initial screening level non-target analysis, followed by targeted analysis of suspected biliary metabolites, using liquid chromatography and high-resolution mass spectrometry. Ex situ rainbow trout liver perfusions were used to quantitatively determine the extent and rate of hepatic biotransformation in the presence and absence of an active transport inhibitor, cyclosporine A (CSA).

Results from the sensitive trout ex situ assays include negligible differences to average hepatic clearance values between control and CSA groups and a high average extraction fraction (> 0.70). These findings indicate a negligible contribution of active transport to hepatic clearance and the presence of rapid biotransformation of 6PPD-Q.

Analytical chemistry of bile samples collected from acutely exposed fishes provided strong evidence for the presence of both hydroxy-6PPD-Q and 6PPD-Q-O-glucuronide metabolites. In vivo bile samples generated cross-species semi-quantitative data

depicting relative quantities of each metabolite suggesting 6PPD-Q is detoxified sufficiently by tolerant fishes but insufficiently by sensitive fishes.

Ex situ results from one sensitive species in conjunction with biliary compounds across species strongly suggests detoxification of 6PPD-Q via phase I hydroxylation as the primary toxicokinetic mechanism for interspecific differences to sensitivities. This insight will help provide future direction to the risk assessment of 6PPD-Q.

### 3.21.P-Tu317 Searching for Transformation Products of 6PPD that are Toxic to the Aquatic Larvae of the Mayfly, *Neocloeon triangulifer*

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The substituted phenylamine compound, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6-PPD) is added to tire rubber as an antioxidant. Recent studies have shown that an oxidation product of 6-PPD, namely 6-PPD-quinone (6-PPDQ) is extremely toxic to Coho Salmon, Brook Trout, and Rainbow Trout, but this compound is less toxic to several other fish species and does not cause mortalities in toxicity tests with the invertebrates, *Daphnia magna* and *Hyalella azteca*. Our toxicity studies with aquatic larvae of the mayfly, *Neocloeon triangulifer* confirmed that 6-PPDQ is not acutely toxic to this insect larvae at concentrations up to its limit of solubility. However, 6-PPD is very unstable in solution and several transformation products in addition to 6-PPDQ are formed by the degradation of this compound. In our 96-h acute toxicity tests conducted with *N. triangulifer* at µg/L nominal concentrations of 6-PPD, the test solution caused mortalities. Targeted analysis of several transformation products of 6-PPD indicated that another transformation product, 4-HDPA was present in the test solution at high concentrations. In tests with 4-HDPA at nominal concentrations between 100 to 800 µg/L, there were mortalities of mayfly larvae of between 30 to 50% but the responses did not closely match predictions based on the 6PPD test. More work is required, but it is possible that a transformation product of 4-HDPA may be causing toxicity. Our subsequent analyses of the test solutions using non-targeted analysis by liquid chromatography with high-resolution mass spectrometry (LC-HRMS) indicate that there are candidate transformation products that could contribute to toxicity to the mayfly larvae.

### 3.21.P-Tu318 Evaluating the Transcriptomic Points of Departure in Early-life stage Rainbow Trout Exposed to 6PPD-quinone

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The rubber tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) found in roadway runoff has recently been implicated as a driver of urban runoff mortality syndrome in coho salmon. Further studies have indicated a highly variable species sensitivity distribution. While classical exposures are integral to determining initial species sensitivity, transcriptomic analysis can provide a broader picture of potential gene expression and biological pathway disruption, as well as providing transcriptomic points of departure (tPODs). An early-life stage toxicity test was conducted on rainbow trout (*Oncorhynchus mykiss*) alevins, beginning at hatch. Treatment groups consisted of a water control, solvent control (0.01% dimethyl sulfoxide), and time-weighted average 6PPD-quinone concentrations of 2.35, 1.30, 0.44, 0.20, 0.10, and 0.06 µg/L. Whole-body alevins were sampled after 96 h of exposure and flash-frozen for transcriptomic analysis. Three individuals were pooled from each replicate, across five replicates per treatment group. RNA was extracted using QIAGEN RNeasy Universal Mini Kits, with a minimum 100ng/µL in 25µL per sample, analyzed by Genome Québec. Analysis using FastBMD will focus on pathway BMDs, and cross-species comparisons.

### 3.21.P-Tu319 Chemicals Leaching from Rubber Microplastics are the Key Drivers of Toxicity Towards Early Life Stages of Atlantic Cod (*Gadus morhua*)

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Methods to accurately assess the toxicity of chemicals in plastics are needed. Importantly, there is a lack of studies successfully deconvoluting particle and leached chemical effects on organisms. A recent investigation by our group indicated elastomer chemicals are more potent than common thermoplastic chemicals in acute toxicity tests. The toxicity of car tire rubber (CTR) leachates and chemicals to marine species have recently been demonstrated in a range of studies, but other rubbers have been less studied. We present a comprehensive study of chemicals leaching from rubber MPs, (rMPs) and their toxicity to cod (*Gadus morhua*) embryos. This study aimed to unravel the role of CTR (rMP model material) as a pollutant particle and as a carrier of chemicals, and thus distinguish between the effects derived from rMPs from those resulting from associated chemicals. To study the influence of experimental parameters on leachate composition, leachates of dishwashing (DG) and lab gloves (LG), balloons (BAL), and CTR were prepared and the effect of particle loading, size, water temperature, salinity and turbulence, as well as leaching time, on the chemical composition of the resulting leachate, were investigated. Early life stages of Atlantic cod (*Gadus morhua*) were used to study the effects of leachates, particles and a combination of the two. First, CTR, DG, BAL leachates were compared in terms of toxicity. Second, we compared 1) CTR particles pre-leached for 30 days to allow desorption of associated chemicals, 2) chemicals leached from the CTR over 7 days, and 3) pristine CTR particles (pCTR) not subjected to

pre-treatment, thus including all associated chemicals. Both experiments were conducted with 4-day exposures, followed by a recovery period of ~11 days. Endpoints included mortality, hatching, developmental alterations, and gene expression. DG and CTR exposure led to lower hatching success and increased mortality compared to BAL leachates, which did not cause any significant effect. Embryonic development was impacted by exposure to CTR, resulting in smaller larvae at hatch. Leachate and pCTR exposures led to significantly lower hatching success and increased mortality compared to pre-leached particles. Larvae exposed to pCTR showed a smaller eye diameter and an increased yolk/body fraction. Only leachates affected eye-to-front distance in a dose-dependent way. Larvae exposed to leachates and pCTR also showed significant reduction in myotome length.

### **3.21.P-Tu320 Identification and Quantification of Tire Wear Particles and Associated Compounds in UK Marine and Freshwater Habitats.**

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The report that shows the toxicity of 6PPD-quinone (6PPD-q), a by-product of a tire manufacturing additive, to coho salmon (*Oncorhynchus kisutch*) has made the discussion of the impact of tire wear particles in aquatic ecosystems a growing environmental concern. Little is known about the specific environmental presence of the particles and associated compounds, but progress has been made with regards to detection. This study seeks to analyse for Tire Wear Particles and some target compounds, 6PPD-quinone (6PPD-q), 1,3-diphenylguanidine (DPG) and 2-mercaptobenzothiazole (MBT) in surface water and sediment samples which were originally collected from River Hamble and Langstone harbour (S. England, UK). Samples were collected in close proximity to major multi-lane highway storm water discharge zones to determine if these compounds were present in stormwater-influenced flows. These particles and target compounds will be analyzed using GC X GC-TOF-MS method. The concentrations of 6PPD-quinone will be compared with the acute LC50 for coho salmon (0.8–1.2 µg L<sup>-1</sup>). Levels of 6PPD-q, DPG and 2-MBT will be determined as well as their limits of quantification in all extracts. Particles will be compared with a reference data base of tire, brake, and road material. This study will contribute to the growing literature indicating that potentially toxic tire-wear particles and compounds are present at elevated levels and are transported via road runoff into urban surface waters during rain events.

### **3.21.P-Tu321 Antioxidants in the European Environment: A Hidden Hazard for Biota?**

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It is assumed that the number of cars worldwide will almost double in the next 30 years: 1.3 billion in 2020 to 2.5 billion in 2050. Tire and road wear particles (TRWP) are formed by mechanical abrasion during road contact. Thus, roads and traffic serve as a major source of land-based microplastic particle pollution.

Tires contain antioxidants that prevent the ageing of rubber by oxygen and ozone. Para-substituted phenylenediamines (PPDs e.g., 6PPD, 7PPD, IPPD, 77PD) are commonly employed for this purpose at concentrations up to 1 to 2%. PPDs are registered in Europe between 1 and 100,000 t / year; the highest registered tonnage in Europe is 6PPD with 10.000 to 100.000 t / year. A CLH dossier for harmonized classification on 6PPD is currently under development.

A recent study identified a transformation product from tires, 6PPD-quinone, which is highly toxic to juvenile silver salmon (24-h-LC50 = 0.095 µg/L). It is responsible for the mass mortality syndrome observed in rivers after heavy rainfall in the USA and Canada. Not only silver salmon are affected, but also species that occur in Europe, such as rainbow trout and brown trout. Some of the substances have a potential to accumulate in organisms, which is proven for 8PPD, for others more research is needed. In general, too little is known about the fate and behavior of TRWP and PPDs including their transformation products.

Our laboratories have developed a method for the determination of various PPDs and the transformation product 6PPD-quinone. Consequently, Austrian surface waters, road run-offs, influents from wastewater treatment plants and freshwater fish were investigated. In none of the 12 investigated surface waters were PPDs detected. In surface run-off following substances were found: IPPD, 6PPD and 6PPD-quinone. In some influents, 6PPD-quinone was found in concentrations higher than the LC50-value for salmon. In one of ten pooled fish samples, 6PPD was above the limit of detection.

In Austria, the amount of TRWP according to calculations is approximately 20,000 tons per year. More research on the occurrence and distribution of TRWP and PPDs including their transformation products in the environment is necessary to set measures for hot spots and to mitigate potential risks for aquatic organisms.

### **3.21.P-Tu322 Leaching of Metals and Organic Compounds from Tires Over Time – Exploring the Effects of Road Pollution Sedimentation Treatment for Tire Leachates**

**Elisabeth Støhle Rødland, Tania Gomes and Muhammad Umar, Norwegian Institute for Water Research (NIVA), Norway**

Road pollution have been studied for decades due to the release of particles and pollutants from road maintenance, vehicles, tires, brakes, and the road surface. Some of the most important road-related pollutants are metals such as zinc, copper, nickel, inorganic minerals, road salts, and organic compounds such polycyclic aromatic hydrocarbons, benzothiazoles, and phthalates. Recent studies have also identified new organic compounds linked to toxic effects in organisms, such as 6-PPD-quinone (N-(1,3-dimethylbutyl)-N'-phenyl-pphenylenediamine, 6-PPD), HMMM (hexa(methoxymethyl)melamine), and DPG (N,N'-Diphenylguanidine). Several have investigated the toxicity of these compounds in different organisms, as well as identified and quantified their levels in different environmental compartments. This study is comprised of three parts where we investigate the leachate level at different times over 21 days (Day 0, 3, 7, 14, and 21). In part 1 we investigated the level of zinc, copper, and nickel and a range of organic compounds (including benzothiazoles, 6-PPDs, HMMM, DPG) in leachates from three car tires (winter studded, winter non-studded, summer) and one truck tire (winter non-studded) at relevant concentrations for tunnel wash water (35 mg/L) in a lab experiment at room temperature. In part 2 we collected samples in a sedimentation treatment basin for

tunnel wash water, where water is treated for 21 days before being released into the environment. These samples will be analyzed similarly to the leachates in part 1. In part 3, the toxicity of the leachates from the different car tires will be assessed using the marine microalgae *Skeletonema pseudocostatum*, focusing on growth inhibition and photosynthetic capacity. Because we only have results for the metals in part 1 so far, the results described are preliminary and will be updated before the conference. For the metals in part 1, the highest levels were found for zinc ( $4.8\text{--}202\text{ }\mu\text{g/L}$ ). The levels between different tire types were also significant, and the highest levels coming from truck tires ( $111 \pm 73\text{ }\mu\text{g/L}$ ). The levels for zinc showed a statistically significant increased with time, with highest levels found at Day 21. For nickel ( $0.10\text{--}1.48\text{ }\mu\text{g/L}$ ) and copper ( $0.20\text{--}2.62\text{ }\mu\text{g/L}$ ), the levels decreased over time, with the highest levels found at Day 0. More statistical analysis will be performed on these data when all analyses are finished.

### 3.21.P-Tu323 Biofilms Can Accumulate and Transform Tire-derived Compounds in Urban Rivers

*Anya Sherman, Thorsten Huffer and Thilo Hofmann, University of Vienna, Austria*

Organic compounds leach from tire wear particles and enter urban rivers around the world at concentrations in the  $\mu\text{g L}^{-1}$  range. N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q) is acutely toxic to several salmonid species at environmental concentrations, and 1'3'-diphenylguanidine (DPG) and benzothiazole (BTZ) also demonstrate toxicity to aquatic organisms. Understanding the environmental fate of tire-derived compounds in urban rivers is crucial. Biofilms are important for the environmental dynamics and fate of organic compounds. Their complex structure, negative charge, and hydrophobic nature allow for sorption of many organic compounds, and their unique microbial communities can biotransform these compounds. We hypothesized that biofilms might accumulate and transform tire-derived compounds in urban rivers. To test this hypothesis, we selected two points where a small urban stream in Vienna receives untreated road runoff and grew biofilms directly upstream and downstream of each point. We brought the mature biofilms to the lab, where we exposed them to N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), 6PPD-q, hexamethoxymethylmelamine (HMMM), DPG, 2-hydroxybenzothiazole (OH-BTZ), and 5-Methyl-1H-benzotriazole (BTR) for 48 h. We monitored all compounds using high-resolution mass spectrometry. Compounds were depleted from the aqueous phase, and taken up into biofilms. Neutral compounds accumulated in biofilms in accordance with their  $\log K_{ow}$  (6PPD-q  $\gg$  OH-BTZ  $>$  HMMM  $>$  BTR). The accumulation of these compounds varied between biofilms, likely due to varying biofilm organic carbon content. Positively charged compounds (DPG and 6PPD) showed generally higher accumulation than the neutral compounds, and accumulation was less biofilm dependent. All compounds accumulated more in biofilms grown downstream of road runoff points, suggesting active uptake in biofilms with previous exposure to tire-derived compounds. Upstream biofilms accumulated tire-derived compounds continuously throughout our experiments, while in downstream biofilms, concentrations increased then decreased, suggesting that downstream biofilms transformed tire-derived compounds, while upstream biofilms did not. The transformation rates varied between the different compounds and biofilms but correlated well with the maximum accumulated concentration. Our data suggests that biofilms accumulate tire-derived compounds and can evolve capacities to transform them.

### 3.21.P-Tu324 Aquatic Ecotoxicity of Safe and Sustainable by Design Organophosphate Flame Retardants

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Organophosphate flame retardants (OPFRs), common polymer additives, were introduced as replacements for polybrominated diphenyl ethers following concerns about environmental and human health effects. However, today OPFRs have established themselves as notorious environmental contaminants. Two widely used OPFRs, tris(2-chloroethyl) phosphate (TCEP) and tris(3-chloropropyl) phosphate (TCPP) are frequently detected in a range of environmental matrices, especially fresh water. Both compounds are classified as very persistent and exert toxicity to aquatic organisms.

To mitigate these adverse effects from the very start of the chemical's life cycle, TCEP and TCPP have been structurally redesigned as a case study of putting the Safe and Sustainable by Design concept into practice. The alternative design is based on a computer-aided framework developed in-house. The aim of the redesign was to retain the flame-retardant properties of the compounds but minimize adverse environmental effects, taking persistent, bioaccumulative, mobile, and toxic properties into consideration. A multicriterial analysis of millions of structures similar to the original chemicals generated in silico, yielded a set of structures predicted to be the most benign, informing our design of structural alternatives for TCEP and TCPP.

To assess the environmental safety of the newly designed compounds, as well as the viability of the computer-aided design approach, the two alternative chemicals were synthesized and together with the original compounds, subjected to a series of aquatic ecotoxicity tests. To gain possible clues about the relationship between ecotoxicological effects and structural trends of OPFRs, we also tested the structural intermediates tris(ethyl) phosphate and tris(propyl) phosphate, which are themselves used as polymer additives. This poster will present the acute and chronic effects of this set of structurally related OPFRs on three aquatic model organisms: algae, daphnids, and chironomids, which are determined following OECD test guidelines. Based on predictions employed during the alternative compound design (USEPA EpiSuite Ecosar), it is expected that in both acute and chronic tests, the proposed alternative compounds are less toxic to all test organisms. The findings of this study will support the Safe and Sustainable by Design concept and precautionary approach including extensive testing of additive chemicals to prevent adverse effects on aquatic ecosystems.

### 3.21.P-Tu325 Property Estimations of Organophosphate Ester Transformation Products: Interpreting Environmental Fate with Chemical Space Plots

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TCPP (Tris (2-chloroisopropyl) phosphate) and EHDP (2-ethylhexyl diphenyl phosphate) are two highly dominate organophosphate ester flame retardants (OPFRs) in air. Numerous transformation products (TPs), produced by heterogeneous atmospheric oxidation, have been identified for both TCPP and EHDP. However, there is limited understanding of the partitioning tendencies of these compounds. The primary objectives of this study were to predict physico-chemical partitioning properties of these two model OPFRs and gain a preliminary understanding of how the environmental fate of both chlorinated and non-chlorinated airborne OPFRs are altered on heterogeneous transformation. However, commercial standards of the TPs are not available, so it became necessary to estimate their physico-chemical properties using computational software. As a result, a secondary objective arose, which was to compare two common property prediction methods, BIOVIA COSMOtherm and the OPEn structure-activity/property Relationship App (OPERA), in terms of their impact on environmental fate assessments for the two suites of OPFR transformation products. OPERA is a popular estimation tool based on quantitative structure–activity/property relationships (QSAR/QSPR), whereas COSMOtherm utilizes quantum mechanisms to calculate the chemical potential of chemicals in solvated environments to calculate macroscopic properties. Using interpretative Chemical Space Plots (CSPs) created with a newly developed RShiny App, it became apparent that heterogeneous oxidation has a significant impact on the partitioning behaviour of the various oxidation products. There is a general decrease in log  $K_{AW}$  with increasing extent of oxidation, leading to a lowering in vapour pressure and increased distribution to aqueous (TCPP) and lipid-rich (EHDP) media. In addition, the agreement between the two computational prediction methods is shown to be best for chemicals that are within the applicability domain of OPERA and are reported by OPERA with high confidence, giving credence to COSMOtherm as a property estimation tool for OPFRs.

### 3.21.P-Tu326 Toxicity of DEHP related to cellular damages and sex-steroid hormone depletion in vitro

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Numerous reproductive issues such as poor semen quality, lower epididymal weight, or increasing incidence of testicular cancer have been related to daily exposure to phthalates. di-(2-ethylhexyl) phthalate (DEHP) is widely used in many industrial applications and is easily released from these products into foods or beverages, which may directly affect human health. Therefore, it is important to identify the exact molecular mechanisms of action and determined the cellular aberrations that could be responsible for decreased reproductive capacity of males. The present in vitro study was undertaken to examine the direct effect of DEHP's treatment on mice Leydig cell line. TM3 cells were cultured and treated with different concentrations (0.05–1 M) of DEHP for 48-h. To determine cell viability and cell membrane integrity, the tetrazolium assay (MTT) and, 5-carboxyfluorescein diacetate, acetoxymethyl ester assay (CFDA-AM) were used. In addition, sex-steroid hormone level fluctuation was quantified by enzyme-linked immunosorbent assay (ELISA). The obtained data clearly demonstrated a dose-dependent effect after the respective treatment. Lower experimental doses of up to 0.25 M of DEHP did not significantly affect cell viability and membrane integrity. Overall, rising experimental doses significantly ( $p < 0.0001$ ) inhibited presented parameters after 48-h exposure. Furthermore, analyses of sex-steroid hormone secretion revealed, that increased experimental doses (starting from 0.15 M) significantly ( $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.05$ ) reduced the testosterone production in TM3 mice Leydig cells. Taken together, the results of our in vitro study reported that higher experimental doses of DEHP could affect cell viability and cell membrane integrity. In addition, experimental concentrations higher than 0.15 M could inhibit steroidogenesis, resulting in a decreased level of testosterone in vitro. A more detailed and systematic research in DEHP toxicology is required for a better understanding of reproductive health risks.

### 3.21.P-Tu327 Leaching and Degradation Kinetics of Legacy and Emerging Plasticisers in Contrasting Soils

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Plasticisers are a widely used class of organic additives designed to increase the flexibility of plastic products. Historically, phthalates have been the most commonly used class of plasticiser, although concerns surrounding the endocrine disrupting properties of some commonly used phthalates have led to increasingly stringent legislation surrounding their production and use in the USA and Europe. This has coincided with an increase in market share of so-called "emerging plasticisers" such as terephthalates, citrates, and adipates.

Although the persistence of phthalates in soils has been fairly well-studied in the laboratory (e.g., common phthalates appear to have half-lives on the scale of weeks to months), the persistence of emerging plasticisers in soils is essentially unstudied (or at least unreported) in scientific literature. This has potential implications for the accumulation of emerging plasticisers in soils and terrestrial biota. In addition, the factors affecting the rate at which plasticisers can leach from microplastics directly into soils represents a further knowledge gap which should be addressed if the impacts of plastic waste on the terrestrial environment are to be fully understood.

We carried out medium-term (3-month) experiments to investigate the leaching and degradation kinetics of legacy and emerging plasticisers in three natural test soils with contrasting properties. The leaching experiment involved spiking soils with PVC microplastic pellets and monitoring the release and degradation of the emerging plasticiser di-ethyl-hexyl terephthalate (DEHTP). The degradation experiment involved spiking the test soils with 8 phthalates and 4 emerging plasticisers. The test soils were the same for both experiments and allow for the comparison of how soil properties affect the leaching and degradation rates of plasticisers, in addition to the comparison of legacy and emerging plasticiser persistence in soils.



Initial results from the leaching experiment suggest that leaching of DEHP from the PVC microplastics directly into soils was rapid, and that the rate of subsequent degradation of DEHP was at least partly dependent on soil pH, with lower DEHP degradation rates in the soils towards the extremes of the pH range found in natural soils. The results from the degradation experiment will represent, as far as the authors are aware, the first scientific study to investigate the degradation rates of emerging plasticisers in soils.

### **3.21.P-Tu328 Role of Soil Organic Matter on the Fate of Common Plastic Additives, Di(2-ethylhexyl) Phthalate, Bisphenol A and Benzophenone, in Soil**

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Plastic additives, for example fillers, plasticizers, UV scavengers, and stabilizers, are being added during the production process to obtain the expected properties such as flexibility and light weight of mulch films. These additives are not chemically bonded with the polymeric chain; therefore, there is a risk of leaching during the degradation and fragmentation of plastics in the environment. Hence, the objective of this study are to understand the factors affecting the retention of selected plastic additives, namely Di-(2-ethylhexyl)-phthalate (DEHP), Bisphenol A (BPA), and Benzophenone (BP). Red yellow podzolic soil was air-dried (24 h) and sieved (2 mm) prior to the experiment. A portion of the soil sample (20 g) was subjected to ignition at 600 °C for 2 h in a muffle furnace to remove the total OM of the soil. The pH edge experiment was conducted at 20 g/L soil suspension spiked with 10 mg/L of each additive (DEHP, BPA, and BP), whereas isotherm experiments were carried out at concentrations of 1 to 20 mg/L of additives spiked into two different soil suspensions (with and without OM). Filtered samples were diluted and analyzed using HPLC. DEHP demonstrated the highest retention, 331 and 194 mg/kg in the presence of OM and absence of OM, respectively, at pH 6.6. However, BPA and BP had their highest retentions at pH 4.2 and 4.4 with soil OM of 90 and 19.5 mg/kg, respectively. The modelling of isotherm data together with FTIR measurements enabled further understanding of the adsorption mechanisms. The results indicate that DEHP was involved in correlative adsorption to soil OM. Yet, physisorption was depicted in soil without OM. BPA was physically bound to soil OM while the mineral component showed strong chemisorption. Nevertheless, BP demonstrated chemisorption on both soil OM and mineral components. The implication of these results suggest that DEHP and BPA could be easily released into soil water; however, BP would most possibly be immobilized in soils.

### **3.21.P-Tu329 Investigating the Effects of Substances Leaching out of FFP2 Masks on the Aquatic Organism *Danio rerio***

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Even if it looks like the COVID-19 pandemic is coming to an end in many countries, its long reign has brought some new challenges with it: an enormous amount of plastic waste generated by the massive, albeit necessary, use of FFP2 and other face masks. Although the use of face masks varied across countries between <15% and 70%, depending on sociodemographic factors and strictness of wearing policies, the number of masks entering the environment has increased during the last years (~17.000 tons are estimated to enter waterbodies per month), due to false disposal. FFP2 masks have been shown to contain substances that exhibit endocrine disrupting properties, such as phthalates. These substances have the potential of leaking out of the masks into the environment.

To test the effects of substances leaking out of FFP2 masks on aquatic organisms, embryos and larvae of *Danio rerio* were exposed to FFP2 leachates. Leachates were obtained by performing a column percolation according to DIN 19528, where 50 g of FFP2 masks (~12 masks) were leached for 7 h using zebrafish system water as mobile phase. Zebrafish eggs (1.5 hpf) were then exposed to leachates in different concentrations (100%–25%) for 96 hpf according to OECD guideline No. 236 to investigate acute toxicity and potentially occurring abnormalities in their development. An LC50 value of 51% and abnormalities occurring at 40% could be observed. Furthermore, locomotor behavior of 96 hpf larvae were determined using a DanioVision observation chamber (Noldus) and tracking software EthoVision XT (Noldus), resulting in altered velocity and distance moved between 25% and 40% exposure. Also, the performance of RT-qPCR with 96 hpf larvae, investigating effects on gene expression of the *vtg1* gene (normalized against *elfa*) compared to 1 µM 17β-estradiol to investigate endocrine effects and a GC-MS detection of the phthalate concentration in the tested leachates are planned.

The results of the experiments indicate that substances could be leached out of the FFP2 masks under conditions similar to the environment (freshwater) and that they show effects on the tested organism, thus posing a potential threat for the aquatic environment. However, detailed ecotoxicological risk assessments that analyze or reliably estimate the actual concentration of substances being leached into the environment (for freshwater as well as for marine waters) must be performed before any conclusion can be drawn.

### **3.21.P-Tu330 Environmental Fate of Plastic Pellets in the Harbour of Antwerp**

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The harbour of Antwerp is the leading polymer hub in Europe for production, handling, and distribution of preproduction plastic pellets. In the marine environment, microplastics, including plastic pellets, are a big source of plastic pollution. Transportation, transformation, and environmental risk are the main factors that determine the fate of released plastic pellets. Exposure of plastic pellets to mechanical stress and environmental conditions can lead to breakdown of the pellet into smaller pieces, leach of additives, changes in physical and chemical properties, absorption of pollutants.

The Port of Antwerp and the companies of the production chain (industry, transport, logistics) are taking measures to reduce pellet losses to the surrounding environment. However, little is known about the efficacy of the measures. So, the question is:

How many pellets are still being released into the environment and where is this occurring? A total of 57 critical points on the public road and 5 accumulation points on the slopes of the docks were intensively monitored using manual sampling site with a quadrant of 50 by 50 cm. The results show a considerable ongoing plastic pellet loss and a high pollution degree on the accumulation points on the slopes of the docks. On the road there was a decrease of the released pellets during 2022. Intensive communication about the research took place with the companies of the plastic value chain, the port authority, and government authorities. Highlighting the problem and sharing the results could be one of the factors that decreased the number of released plastic pellets.

Despite the increasing awareness, there is still not much known about the effect of plastic pellet pollution on biota in the surrounding area, i.e., the dock water and the Scheldt estuary. Because of trade secrets, there is not much known about the exact recipe and additives of the plastic pellets. After gaining the confidence of the companies by open communication about the research, in 2023 we will set up an in situ experiment in the port, under relevant environmental conditions, with relevant plastic pellets as they are produced in the same area. New plastic pellets will be exposed to environmental conditions (terrestrial, aquatic), samples will be taken and analysed to identify physical-chemical modifications occurring. The aim of the study is to build knowledge and perspective to better understand the environmental risk of a released plastic pellet and its additives.

### **3.21.P-Tu332 Leachate Effects in Biodegradable Geotextiles**

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In recent years, a development from petroleum derived synthetic non-biodegradable polymers to biobased and biodegradable polymers can be observed in various sectors, amongst them the agricultural, consumer product, and building sectors. The latter stands out due to its direct contact and implementation into the environment, but also its large-scale application. A widely used application in this field is geotextiles, which can be found integrated into different soil layers, on top of them or even above with only a small contact surface. It is therefore highly commendable to develop a sustainable alternative to conventional products that guarantee stability and function as well as environmental compatibility. One crucial factor in developing an environmentally friendly polymer is their composition, which ideally should not pose a risk to the environment due to, for example, additives. Critical additives, which for example pose threats of mutagenicity, endocrine activity, reproductive toxicity, or accumulation, may be released during use of these applications. This risk needs to be prospectively screened to assess the overall impact of geotextiles. In our project, we want to examine 4 different biodegradable geotextiles and optionally petroleum derived equivalents in terms of potential leaching of substances in accordance with DIN EN 16637-2. After eluting the materials in water for 6h, 1 and 36 days, the eluates will be investigated in different biotests. Hereby mutagenicity via micronucleus assay, dioxin-like activity via micro EROD, and acute aquatic toxicity via *Daphnia* toxicity test are screened with potential addition of other test systems after positive findings. First results from these experiments will be presented.

### **3.21.P-Tu333 Leaching of Endocrine Disrupting Chemicals from Plastic Food Packaging – In Vitro Toxicity and Chemical Composition**

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Plastics are chemically highly complex materials. More than 10,000 substances are used in plastic production (e.g., plasticizers, antioxidants) and even more non-intentionally added substances (e.g., reaction byproducts, impurities) are associated with them. While there is abundant scientific evidence that some compounds used in plastics, such as bisphenols and phthalates, are endocrine disrupting chemicals, little is known about the composition and toxicity of the complex mixtures of chemicals leaching from everyday plastic products. Therefore, this study aims at characterizing the in vitro toxicity and the chemical composition of leachates from plastic food contact articles (FCAs) as relevant sources of human exposure. Fourteen plastic FCAs covering the 7 polymer types with the highest global market share were leached into water and a water-ethanol mixture for 10 days at 40°C according to European Regulation 10/2011/EU. The leachates were analyzed with reporter gene assays for a set of nuclear receptors relevant to human health, including pregnane X receptor (PXR), peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), estrogen receptors alpha (ER $\alpha$ ), and androgen receptor (AR). Further, these plastic mixtures were analyzed using non-target high-resolution mass spectrometry to quantify the number of chemical features and tentatively identify the chemicals leaching from the FCAs. Thirteen of the fourteen FCA leachates interfered with at least one of the nuclear receptors. PXR and PPAR $\gamma$  were the predominant targets of the plastic chemicals, but both steroid receptors were also activated or inhibited by several samples. The water leachates were less toxic than the water-ethanol leachates and none of the latter was estrogenic. Also, lower numbers of chemicals were leaching into water as compared to water-ethanol mixture. To identify the active compounds in these complex mixtures, an effect-directed analysis will be performed. This research shows that plastic FCAs leach endocrine disrupting chemicals into food simulants and highlights the importance of analyzing whole mixtures of finished plastic products to cover the toxicity of unknown compounds and mixtures.

### **3.21.P-Tu334 Establishing a Database of Plastic Additives Used in Commerce and Their Regulatory Status Across the Globe**

*John Norman, American Chemistry Council*

The International Council of Chemical Associations (ICCA) is engaging with the global community to contribute to the ongoing discussion regarding plastics in the environment and circularity. Attention to plastic waste has grown and global efforts are underway to reduce and prevent its entrance into the environment. With this increase attention on plastics, there is also an increased focus on the additives used in plastic materials.

Additives are substances intentionally added to plastics that impart functional, fit for purpose characteristics to the plastics. Additives improve, modify, or retain plastic properties such as providing flexibility or stability during the plastic life cycle. Without additives, plastic materials would have limited applications, be brittle, and potentially degrade prematurely making them unsuited for critical uses (e.g., safety equipment, medical devices, etc.). Certain additives are designed to enable plastics of different qualities to be blended, increasing the ability to use recycled plastic for products and packaging. Additives are typically classified and categorized by the function or purpose of their use (e.g., antioxidants, impact modifiers, heat stabilizers, etc.). One potential barrier to innovating new technologies and reduce and eliminate plastic waste is the lack of a comprehensive database containing the composition of additives by polymer type, by application, and the relevant safety information.

ICCA is developing a searchable database to allow users to determine whether a substance is currently being used as a plastic additive, what type of application it is being used for, and whether that substance – application combination is currently assessed and/or regulated by relevant governmental agencies across the globe. A vast amount of information has been generated for the polymer type being used and for many of the chemicals used as additives. Regulatory programs like TSCA in the USA; CEPA in Canada; REACH in the EU; K-REACH in Korea; CSCL in Japan; and AICIS in Australia are only a few of many that have evaluated materials for safe use. Assembling all this information in one place will allow environmental modelers and bench scientists to design targeted research to close information gaps. This presentation will give an update on the progress of the project and give attendees an opportunity to engage with researchers to ensure the database meets the needs of the scientific community.

### **3.21.P-Tu336 Evaluating Migration of Polymer Additives Under Environmentally Relevant Conditions – Validation of Modeling Approaches and Application for Product Classification & Labeling under EU CLP & UN GHS**

**Craig Warren Davis<sup>1</sup>, Cynthia Mitchell<sup>2</sup> and David Simpson<sup>3</sup>, (1)Toxicology & Environmental Science, ExxonMobil Biomedical Sciences, (2)Technology Division, ExxonMobil Product Solutions, USA, (3)ExxonMobil Chemical Company**

With inclusion of polymers within the scope of REACH (EU & Korea), efforts to characterize the potential migration of additives, oligomers, monomers, and non-intentionally added substances (NIAS) as well as their relevance for grouping and data generation for registration have increased considerably. Technical limitations as well as the considerable cost associated with method development for additive chemistries and their potential transformation products necessitate an approach which combines systematic experimental data generation with physical-chemical property estimation and migration modeling. While specific guidance for data generation as well as migration modeling is established for specific use cases (e.g., food contact applications), to date, limited data and practical guidance is available for environmentally relevant systems (e.g., aquatic toxicity test systems). Novel data is presented quantifying migration potential of 9 common polymer additives (and identified transformation products) across a broad range of polymer matrices (PE, PP, PVA-copolymers). Migration was then compared to modeling results obtained assuming equilibrium partitioning (EqP) in the test system as well as results from USEPA's AMEM model (which incorporates diffusion, partitioning and surface mass transfer limitations). Results were also compared, where available, to migration studies performed under food contact relevant conditions. In addition, migration of low molecular weight oligomer materials from several grades of adhesives was quantified using biomimetic extraction techniques (BE-SPME) and compared to experimental ecotoxicity thresholds established for the BE-SPME technique. Finally, migration data were used to estimate potential exposure of aquatic organisms to additives under environmentally-relevant conditions (30-d chronic exposure in fresh and seawater). Predicted environmental concentrations (PECs) were computed and compared to experimental and modeled toxicity thresholds for the materials (e.g., PNECs) and risk characterization ratios (RCRs) were computed. The combination of data generation and modeling can be used to support efficient grouping and data generation for polymer categories under EU REACH as well as to support existing and future classification and labeling of finished polymers under EU CLP and UN GHS.

### **3.21.P-Tu337 Toward a Framework for Rapid, Tiered Risk Assessment, & Prioritization of Additives, Impurities, and non-Intentionally Added Substances (NIAS) in Polymers & Plastic Articles**

**Craig Warren Davis<sup>1</sup>, Allison L Isola<sup>1</sup>, Laura Maurer<sup>1</sup> and David Simpson<sup>2</sup>, (1)Toxicology & Environmental Science, ExxonMobil Biomedical Sciences, (2)ExxonMobil Chemical Company**

With the inclusion of polymers within the scope of REACH as well as UN efforts surrounding additives in polymers and plastic articles, efforts to identify and prioritize substances of potential concern has increased substantially. While attempts have been made to prioritize additives, impurities, and non-intentionally added substances (NIAS), these approaches often focus on high-level hazard properties (e.g., GHS classification) and minimize or ignore physical-chemical properties which govern fate and transport of these materials between polymer matrices and their surrounding environments (i.e., exposure). Further, limitations of chemical identifiers (e.g., CASRN) to adequately capture availability of categorical and structural-analogue data presents a challenge to reliably prioritizing truly data-poor substances against those which may be adequately evaluated via read-across or weight of evidence (WoE) approaches. The combination of "hazard-only" frameworks and incomplete data collection may result in a significant over-estimation of chemistries for which adequate data to quantitatively evaluate risk is not available.

The purpose of this work is three-fold: (1) A systematic workflow for the collection and synthesis of in-silico, in-vitro, and in-vivo hazard data for chemicals is proposed which leverages existing QSAR models, experimental databases, and existing regulatory evaluations (e.g., GHS, TDI/ADI values). (2) A systematic workflow for the collection and synthesis of environmental fate, migration, uptake, and exposure for aquatic organisms as well as man exposed via the environment (MvE) is proposed which provides a basis for establishing predicted environmental concentrations (PECs) and human oral dose estimates to be used in quantitative risk assessment and prioritization. (3) A tiered framework is proposed which incorporates both exposure and hazard

information to develop quantitative and categorical assessments of risk, which can be used to effectively prioritize and screen additives and associated chemicals in a consistent and transparent way.

The utility of the framework is demonstrated using a database of polymer and plastic-associated chemistries ( $n = 4756$ ) developed by the Food Contact Forum (FCF). Risk characterization ratios (RCRs) as well as data completeness metrics are used to prioritize and evaluate the database of chemistries. Finally, this prioritization approach is compared to those developed by FCF, ECHA, and others.

### 3.21.V Polymer Additives and Their Transformation Products as Chemicals of Emerging Concern: Environmental Emissions, Fate Processes, and Impacts

#### 3.21.V-01 Additive chemicals in plastic marine debris and fishing gears

**Mi Jang<sup>1</sup>, Youna Cho<sup>2</sup>, Gi Myung Han<sup>3</sup>, Sung Yong Ha<sup>4</sup> and Sang Hee Hong<sup>2</sup>**, (1)Risk Assessment Research Center, Korea Institute of Ocean Science and Technology (KIOST), Korea, (2)Korea Institute of Ocean Science and Technology (KIOST), Korea, (3)Risk Assessment Research Center, Korea Institute of Ocean Science and Technology (KIOST), Korea, (4)Risk Assessment Research Center, Korea Institute of Ocean Science and Technology (KIOST), Korea Plastic marine debris can remain in the ocean for long periods of time. Plastic products contain numerous additive chemicals, which are present in large amount (~70% by weight) and can be transferred to the marine environment. In this study, additive chemicals and persistent organic pollutants (POPs) were quantified in marine plastic debris and fishing gear, along with their corresponding new plastic products in markets, categorized by fisheries ( $n = 35$ ), package ( $n = 12$ ) and cigarettes ( $n = 7$ ). Additive chemicals significantly higher than POPs in plastic samples (t-test,  $p < 0.05$ ), indicative of high use of additives in plastics. New products contained a significantly higher amount of additives than debris samples (t-test,  $p < 0.05$ ), implying their potential leaching or degradation during use or after disposal in the marine environment. In the fisheries category, buoys generally contained high concentrations of antioxidants (0.28–91  $\mu\text{g/g}$ ) such as 2,4-di-tert-butylphenol (2,4-DTBP) and butylated hydroxytoluene (BHT), Irgafos168, Irganox1010, and Irganox1076. Among the buoy, polyurethane covered buoy had a high amount of phthalate such as Di-(2-ethylhexyl) phthalate (DEHP; 47  $\mu\text{g/g}$ ). Black colored polypropylene (PP) type of buoy contained high concentration of ultraviolet stabilizers (UVs; 4.3  $\mu\text{g/g}$ ) and brominated flame retardants (BFRs) such as BDE 209 (3.5  $\mu\text{g/g}$ ) and DBDPE (200  $\mu\text{g/g}$ ). Polyvinyl chloride (PVC) type of rope used for aquaculture farm had a highest concentration of phthalate (DEHP, 520  $\mu\text{g/g}$ ) and biphenyl A (BPA; 101  $\mu\text{g/g}$ ), and the second highest concentration of UVs (1.4  $\mu\text{g/g}$ ). Polyethylene (PE), polypropylene (PP), polyester (PES), and polyamide (PA) types of ropes, eel trap (PP) and net (PE) for fisheries generally had a low concentration of additive chemicals compared with buoys. Rubber bars from tires for aquaculture farms contained the highest concentration of tire antioxidant (6PPD-quinone; 11.4  $\mu\text{g/g}$ ), which is the toxic chemical responsible for killing salmon. Among the package category, plastic bag had a high number of antioxidants (2,4-DTBP, 11.5  $\mu\text{g/g}$ ) and BFRs (BDE209, 5.2  $\mu\text{g/g}$ ). Targeted additive chemicals were detected in the lowest concentration in all cigarette samples. The present study provides quantitative information about additive chemicals contained in plastic marine debris and their products. Further study of release, transfer, and accumulation of these additive chemicals from plastic debris to marine environment is needed.

#### 3.21.V-02 Environmental Monitoring of 6PPD-quinone Present in Stormwater Across Canadian Cities

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*N*-(1,3-Dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), a transformation product of the rubber tire antioxidant 6PPD found in road runoff, has been found to be acutely toxic to some species of fish. While previous studies have shown the great importance of stormwater as a source of 6PPD-quinone, less is known about environmental and land-use drivers, and spatio-temporal dynamics across a larger study area. Therefore, this study aims to assess the levels of 6PPD-quinone in stormwater from three Canadian cities: Calgary, Edmonton, and Lethbridge. Stormwater samples were collected from these cities throughout May to October 2022 and stored frozen. Samples were extracted using solid phase extraction (SPE) and 6PPD-quinone quantified using Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS). Preliminary analyses show a range from 4.03 ng/L to 1.23  $\mu\text{g/L}$  of 6PPD-quinone in stormwater samples from the City of Calgary. Because the median lethal concentrations (LC50s) of 6PPD-quinone in brook trout and rainbow trout, two species found in the receiving water bodies, are 0.59 and 1.00  $\mu\text{g/L}$ , respectively, the presence of 6PPD-quinone in stormwater might affect aquatic ecosystem health adversely. This research will provide information that will help improve the ecological and environmental risk assessment of this important contaminant of emerging concern.

### 3.22.A POPs and Emerging Pollutants: Environmental Fate, Research, and Monitoring at the Interfaces of Science and Policy and Application of Green Removal Technologies

#### 3.22.A.T-01 What Do We Know about the Production and Release of Persistent Organic Pollutants in the Global Environment?

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Humans have manufactured and commercialized huge quantities of persistent organic pollutants (POPs) for various agricultural, industrial, and consumer applications. However, these substances have been revealed to be persistent, bioaccumulative, toxic, and ubiquitous in the global environment. Information on the global production and environmental releases of POPs is of critical

importance for regulating and eliminating these chemical substances of worldwide environmental and health concerns. In this presentation, we report our efforts of a comprehensive literature review of quantitative information on the historical global production and multimedia environmental releases of 25 intentionally produced POPs. Our assembled data indicates that as of 2020, a cumulative total of 31 306 kilotonnes (kt) of the 25 POPs had been synthesized and commercialized worldwide, resulting in cumulative releases of 20 348 kt into the global environment. As of 2020, short-chain chlorinated paraffins were the most produced POP, with a historical global cumulative tonnage amounting to 8795 kt, whereas  $\alpha$ -hexachlorocyclohexane (HCH) had the largest historical global cumulative environmental releases of 6567 kt among these 25 POPs. The 1970s saw the peak in the annual global production of the 25 investigated POPs. The United States and Europe used to be the hotspots of environmental releases of the 25 investigated POPs, notably in the 1960s and 1970s. By contrast, global environmental releases occurred primarily in China in the 2000s–2010s. Overall, our work for the first time reveals the magnitude of global historical production and environmental releases of POPs. We encourage global joint efforts of high-quality data on chemical production, trade, use, waste management, and environmental releases. Such efforts necessitate (i) more comprehensive collection and curation of first-hand national and regional statistical data under the Stockholm Convention and other multilateral environmental agreements, and (ii) global high-level synthesis, comparison, and evaluation of the national and regional statistical data to ensure their transparency, completeness, consistency, and accuracy.

### 3.22.A.T-02 Persistent Problem: The Global Challenges to Managing PCBs

**Lisa Melymuk<sup>1</sup>**, Jonathan Blumenthal<sup>2</sup>, Ondrej Sanka<sup>1</sup>, Veena Singla<sup>3</sup>, Adriana Shu-Yin<sup>4</sup>, Katerina Sebkova<sup>1</sup>, Kristi Pullen Fedinick<sup>5</sup> and Miriam L. Diamond<sup>4</sup>, (1)Recetox, RECETOX, Masaryk University, Czech Republic, (2)University of Toronto, Canada, (3)National Resources Defense Council, United States, (4)Department of Earth Sciences, University of Toronto, Canada, (5)Natural Resources Defense Council, Washington D.C., USA

Polychlorinated biphenyls (PCBs), “famous” as persistent organic pollutants (POPs), have been managed since the 1970s, and the Stockholm Convention on POPs requires environmentally sound management (ESM) of PCBs by 2028. Yet globally, we are not expected to meet this deadline: at best, 30% of countries are on track to achieve ESM by 2028. Over 10 million tonnes of PCB-containing materials remain, mostly in countries lacking the ability to manage PCB waste. The Stockholm Convention guidance and deadlines have proven effective in many high-income regions: Canada (Ontario) and Czechia, both parties to the Stockholm Convention, are close to achieving the 2028 goal, having reduced their stocks of pure PCBs by 99% in the past 10 years. In contrast, the USA, the world’s largest producer and consumer of PCBs, is not a party to the Stockholm Convention, and continues to have a substantial but poorly-inventoried stock of PCBs, with only minimal reductions since 2006. The failure to manage global PCB stocks >30 years after the end of production highlights the urgent need to prioritize reducing production and use of newer, more widely-distributed POPs, such as chlorinated paraffins and per- and-polyfluorinated alkyl substances, because these management challenges are unlikely to be resolved in the coming decades.

### 3.22.A.T-03 Contamination of Mine Water Effluents by Polychlorinated Biphenyls (PCBs)

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Polychlorinated biphenyls (PCBs) are among the persistent organic pollutants (POPs) that have been globally banned since 2001 due to their persistence, bioaccumulation, and chronic toxicity. Manufactured as industrial chemicals, PCBs were used in pure form or as an admixture, mainly as insulating oils in transformers and capacitors. In underground mining, they were even required by fire safety regulations. Due to its high salt concentrations and associated high loads of metals and metalloids, mine water represents one of the world’s largest pollutants streams in terms of volume. To date, mainly inorganic pollutants are considered and treated. However, as a legacy of mining, (abandoned and closed) mines may contain relevant quantities of PCBs. Mine water must be pumped out in most circumstances and is discharged into receiving water courses. Where monitoring of PCB contamination takes place, it is mainly targeted at receiving waters. Of the PCBs with theoretically 209 congeners, the six indicator PCBs and one dioxin-like PCB are normally considered in analysis. Therefore, the aim of the present study was (i) to develop a simple, rapid, miniaturized, and solvent-free method with low detection and quantification limits for PCB congeners analysis in mine water and mine water-affected rivers, and (ii) to elucidate both congener-specific patterns and their time-dependent changes within specific mines by determining PCB concentrations and annual loads. For PCB analysis, a solid-phase microextraction (SPME) method combined with gas chromatography-mass spectrometry (GC-MS) was optimized for the extraction and detection of several PCB congeners directly from untreated mine water. As a result, more than 50 PCB congeners could be quantified in the samples from five different mines in Germany, some over a period of 3 years. The presented method allows a comprehensive and labor-saving analysis of PCBs even in the smallest amounts of 10 mL of untreated, matrix-rich mine water, with very low detection limits (LOD 0.005–0.58 ng L<sup>-1</sup>). The PCB concentrations and annual loads (80–660 g a<sup>-1</sup> per mine) indicate that mine water is an additional source of PCBs in the environment. Because PCB discharge occurs at point sources, targeted water treatment is recommended to achieve a load reduction.

### 3.22.A.T-04 Multimedia Assessment of Legacy and Current Use Halogenated Flame Retardants in Air, Precipitation, Herring Gull Eggs and Lake Trout in Lake Ontario

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In support of the Canada/US Great Lakes Water Quality Agreement and the Stockholm Convention on Persistent Organic Pollutants (POPs), polybrominated diphenyl ethers (PBDEs) and alternative halogenated flame retardants (HFRs) were measured in air (2005–2017), precipitation (2006–2018), lake trout (1997–2019) and herring gull eggs (2008–2018) in the Great Lakes region. All targeted HFRs were detected in one or more air [detection frequency (DF): 0.80–98%], precipitation (DF: 6.4–97%), lake trout (DF: 34–100%), and herring egg (DF: 8.3–100%) samples throughout the sampling periods. Temporal analysis showed declining trends for PBDEs in air, precipitation and lake trout since mid-2000, while herring gull eggs showed little or no change in trends. Non-PBDE HFRs do not show clear temporal trends. Hexabromocyclododecane (HBCDD) was the only non-PBDE HFR that was detectable in all 4 media. Concentrations of HBCDD decreased from 2006 to 2011 but started to increase after 2011 both in air and precipitation. On the other hand, HBCDD showed no trends in lake trout and herring gull eggs. While atmospheric concentrations of HFRs are directly impacted by emissions from sources, concentrations in biota are influenced by many other factors including changes in diet, chemical bioavailability, metabolism, and excretion rates; thus, may result in differing trends and a time lag in our ability to detect any change in trends. These multimedia long-term datasets were combined with a multimedia environmental model, the modified Quantitative Water Air Sediment Interaction (QWASI) model, based on site-specific environmental parameters from Lake Ontario to better understand the observed trends and environmental fate of HFRs in the region. Model results suggest that the loadings from tributaries and wastewater effluent were the primary sources for PBDE and HFR input to the lake. Total degradation in the water and sediment were the dominant removal pathway for BDE 47, 209 and HBCDD, followed by sediment burial.

### 3.22.P-Mo238 Halogenated Flame Retardants in Irish Waste Polymers: Concentrations, Legislative Compliance, and Preliminary Assessment of Temporal Trends

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Halogenated flame retardants (HFRs) were measured in 470 waste plastic articles from Ireland between 2019 and 2020. We identified articles containing concentrations of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD), and tetrabromobisphenol-A (TBBP-A) exceeding European Union limits. Other HFRs targeted were detected infrequently and predominantly at very low concentrations. However, 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ) was detected in 3 display/IT product samples at 14,000 to 32,000 mg/kg, indicating elevated concentrations of FRs used as alternatives to PBDEs and HBCDD, will likely increase in future. Comparison with data for Ireland in 2015–2016, revealed concentrations and exceedances of limits for PBDEs, HBCDD, and TBBP-A were similar or have declined. For end-of-life vehicle fabrics and foams, HBCDD and  $\Sigma$ PBDE concentrations declined significantly ( $p < 0.05$ ) since 2015–2016. Moreover,  $\Sigma$ PBDE concentrations in waste small domestic appliances are significantly lower in 2019–2020, with a similarly significant decline for TBBP-A in waste IT and telecommunications articles. In contrast, HBCDD concentrations in waste extruded polystyrene increased significantly between 2015–2016 and 2019–2020. For other waste categories studied, no statistically significant temporal trends are evident ( $p > 0.05$ ). Fewer samples exceeded PBDE and HBCDD limits in 2019–2020 (7.8%) than 2015–2016 (8.7%), while exceedances for TBBP-A fell from 2.4% in 2015–2016 to 0.57% in 2019–2020. Enforcement of existing limits of 1000 mg/kg will render an estimated 3.1% (2800 t) of articles in the waste categories studied unrecyclable, increasing to: 4.0, 4.9, and 5.6% if limits were reduced to 500, 200, and 100 mg/kg respectively. Meanwhile, enforcing limits of 1,000, 500, 200, and 100 mg/kg will respectively remove 78, 82, 84, and 85% of PBDEs, HBCDD, and TBBP-A present in such waste. While comparison between the 2015–2016 and 2019–2020 datasets provide a preliminary indication of changes, further monitoring is required if the impact of legislation designed to eliminate HFRs from the waste stream is to be fully evaluated.

### 3.22.P-Mo239 Assessment of the Risk Caused by PCBs, PAHs, PCDD/Fs, and PFAS in Bio-based Fertilizers (BBFs) from Various Waste Origins and Obtained Through Different Valorisation Methods

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Agriculture strongly relies on imported mineral phosphate and nitrogen fertilizers whose production is energy intensive and depletes natural resources. Increasing the usage of bio-based fertilizers (BBFs) can allow the reduction and dependence on mineral fertilizers and close nutrient loops. However, persistent organic pollutants (POPs) including PFAS potentially present in organic waste risk ending up in the BBFs, and thus in the environment. Few studies have investigated the presence of PFAS and other POPs in BBFs and assess the risk they constitute for soils, plants and humans. To fill this gap, several classes of POPs – i.e., PCBs, PAHs, PCDD/Fs, and PFAS – were quantified in BBFs from various waste origins (e.g., plants, animal, sludge) and produced through different valorisation methods (e.g., calcination, pyrolysis, hygienisation); then the risk caused by the BBF application to soils, plants, and consumers was assessed.

Nineteen BBFs representative of different risk levels were chosen based on the waste origins and the valorisation methods. Total concentrations of POPs were measured by gas or liquid chromatography coupled with tandem or high-resolution mass spectrometry (GC/MSMS, GC/HRMS or LC/MSMS) after solvent extraction. The POP concentrations expected in amended soils and plants were calculated using recommended/regulated application rates of fertilizers and bioaccumulation factors (BAFs). For most of the BBFs, the concentrations of organic contaminants were (much) below the (few) existing (national) limit values (200 ug/kg for  $\Sigma$ 7 iPCBs, 6 mg/kg for  $\Sigma$ 16 PAHs, 100 ngTEQ/kg for PCDD/Fs, 100 ug/kg for  $\Sigma$ PFOS and PFOA). However,

certain valorisation methods (e.g., hydrothermal conversion) or types of waste (e.g., animal waste) were shown to be more sensitive, leading to high concentration of PAHs (up to 6.9 mg/kg) and PFAS (up to 73.3 ug/kg) in some BBFs. Calculated concentrations in soils fell in the background contamination levels, which is good news to the large-scale implementation of BBFs and the closure of nutrient cycles in the EU. To substantiate this conclusion, the next project activity will be to compare the calculated concentrations in soils and plants to those measured in samples from field trials.

### **3.22.P-Mo252 The Impact of Sediment Turbidity on PAH Resuspension and Potential Baseline Toxicity**

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Sediments store a wide range of contaminants, particularly hydrophobic ones, such as PAHs. Under high turbulence, sediment particles are resuspended in the water column, where chemicals are released. In the water phase, the freely dissolved concentration ( $C_{free}$ ) represents the bioavailable fraction of the chemical. This study aims to understand how sediment resuspension affects PAH leakage from sediment to the water and how this leakage is modulated by PAH hydrophobicity. An experiment was carried out with cores of spiked sediment with 4 PAHs (acenaphthene, fluorene, phenanthrene, and fluoranthene; 3 mg kg<sup>-1</sup>) with a gradient in log Kow from 3.9 to 5.2. A polyethylene passive sampler (PE) was added to each core, and different flow velocities were applied to create different resuspension levels. The turbidity was measured and used as a proxy for sediment resuspension. PEs were collected after 2, 3, and 4 weeks of exposure and PAHs were extracted from the PE and quantified by GC-MS. The chemical activity (the effective concentration of a chemical in a compartment, e.g., water) was calculated for each PAH and summed to estimate the baseline toxicity of the mixture.

The turbidity increased with the flow velocity but decreased after two weeks of exposure, probably due to particle aggregation. The uptake of PAHs by the PE was affected by turbidity and the molecule's hydrophobicity, with a significant interaction between the two variables. At low turbidities (<3 NTU), PAHs were accumulated according to their gradient of hydrophobicity with higher accumulation for lower Kow. However, as turbidity increased, the uptake of congeners with different Kow converged to become similar at >7 NTU. These dynamics are most likely driven by PAH diffusion and desorption from the resuspended particles at low and high turbidities, respectively. Finally, a model ensemble was established to (1) predict  $C_{free}$  using turbidity and logKOW values from the experiment and (2) estimate the potential baseline toxicity of the mixture from the  $C_{free}$  values. Our findings show that using passive samplers in field surveys should be combined with turbidity measurements to obtain PAH concentrations (and other hydrophobic contaminants) under environmentally relevant conditions. Thus, this study contributes to improved hazard assessment of pollutants in the sediments and demonstrates the need to account for the natural variability of resuspension in the environment.

### **3.22.B POPs and Emerging Pollutants: Environmental Fate, Research, and Monitoring at the Interfaces of Science and Policy & Application of Green Removal Technologies**

#### **3.22.B.T-01 A Combined Time-Trend and Trophic Magnification Study on POPs and Emerging Contaminants in a Baltic Sea Food Web**

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Many chemicals that were discovered, synthesized on an industrial scale, and used in everyday life according to the needs of humankind during the 19<sup>th</sup> and 20<sup>th</sup> centuries, like the pesticide DDT, were later shown to be responsible for declining numbers in the various Baltic species populations. These were classified as anthropogenic hazardous substances (AHSs) and investigated further. Many AHSs are toxic and, due to their fat-soluble nature, can be stored in fatty tissues. Moreover, they magnify in species at high trophic level (predator) of the food web, as a result of persistence and transfer from the lower-level species (prey). This transfer is called trophic magnification and is characterized by trophic magnification factor (TMF). AHSs can be divided into known contaminants (e.g., persistent organic pollutants (POPs), such as DDT and PCBs) and contaminants of emerging concern (CECs), including various polymer additives, flame retardants, and others. Some AHSs have been regulated since the 1970s. To evaluate whether the regulations are successful, a retrospective analysis of the samples from different years, a time-trend study, characterized by annual change value (AC, in %), is used. In this work, a non-target screening (NTS) approach was utilized to find and identify a wide variety of chemicals (POPs and CECs) and assess their time trends and/or trophic magnification. An efficient gas chromatography–high-resolution mass spectrometry (GC-HRMS)-based NTS workflow (including sample extraction, clean-up, and instrumental analysis) was established. It was accompanied by a new highly-automated data processing NTS workflow. Both workflows were then applied to a set of Baltic biota samples, revealing time-trend and biomagnification data for a plethora of organic contaminants in a given Baltic Sea food web: >600 identified and unknown compounds showed significant temporal trends and >500 showed significant trophic magnification. A number of CECs were reported for the first time in environmental biota samples. Nowadays, the legacy POPs show decreasing time trends meaning the restrictions work. The workflows developed and data acquired can contribute to the NTS analytical tool development and to the assessment of the influence of AHSs on the ecosystem, respectively. Furthermore, the outcome of these studies can be used in various mitigation actions on AHSs.

#### **3.22.B.T-02 Passive Air and Water Sampling in High-Mountain Lakes: Distribution of Legacy and Emerging Organic Pollutants**

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Remote high mountains allow the study of the environmental fate of legacy and emerging anthropogenic semi-volatile organic compounds like polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organophosphate esters (OPEs), and other organochlorine compounds like hexachlorobenzene (HCB) that have become ubiquitous through long-range atmospheric transport. Passive samplers provide significant benefits for studying remote sites: low cost, ease of use, no energy supply required, and time-integrated assessment of average concentrations. Here, polyurethane foam and low-density polyethylene were used in air and water from six alpine lacustrine areas (1619–2453 m) in the Pyrenees, Spain.

In air, the mean concentrations of  $\Sigma$ PCBs, HCB, and PeCB,  $13 \pm 4$  pg/m<sup>3</sup>,  $44 \pm 18$  pg/m<sup>3</sup>, and  $23 \pm 20$  pg/m<sup>3</sup>, respectively, were similar to those measured 20 years ago in the same area, showing the persistence of these compounds.  $\Sigma$ PAHs was  $631 \pm 238$  pg/m<sup>3</sup>, half of those measured 20 years ago, which agrees with reductions in European atmospheric emissions.  $\Sigma$ OPE concentrations,  $16$ – $53$  pg/m<sup>3</sup> on average, were measured for the first time in this area. Statistically significant temperature dependences of the gas phase concentrations of many compounds revealed the prominence of secondary emission sources (e.g., soils, snow) for OPEs and the heavier PCBs and PAHs, as their lower volatilities cause them to partition more prominently towards other environmental compartments. HCB and the lighter PAHs and PCBs did not present significant changes with temperature, as expected from their higher volatility.

In water, HCB concentrations ( $1.0$ – $14$  pg/L) remained essentially the same as those measured over two decades ago.  $\Sigma$ PAHs ( $35$ – $920$  pg/L) were around half of those observed in the past.  $\Sigma$ PCB concentrations ( $1.2$ – $2.2$  pg/L) were substantially lower, although unexpectedly large differences could be due to comparing yearly averages to seasonally variable episodic pumping measurements from previous studies.  $\Sigma$ OPEs were  $139$ – $2849$  pg/L. Diffusive exchange fluxes between air and water showed deposition of actively emitted PAHs, near-equilibrium for most PCBs and OPEs, and slight volatilization of HCB. In its current state, this high-mountain region may be acting as a sink for several pollutants that accumulate in its surfaces and waters, including some of emerging concern, but some compounds may reach equilibrium between air and other compartments and experience seasonal secondary emissions.

### 3.22.B.T-03 The Atmospheric Fate of TBEC: Spatial Patterns, Seasonal Variability, and Deposition to Canadian Coastal Regions

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As traditional BFRs are gradually phased out of production and usage internationally, emerging brominated flame retardants (EBFRs) have been increasingly produced and used in recent years, with little to no environmental assessment or regulation. Despite its repeated global detection, concerted efforts in monitoring the air concentrations and atmospheric deposition of the EBFR, 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBEC), in Canada have been limited. Moreover, its potential environmental behaviour is still poorly understood, particularly any differences in its two main isomers,  $\alpha$ - and  $\beta$ -TBEC. With the objective to gain insight in the atmospheric fate of  $\alpha$ - and  $\beta$ -TBEC in general and in Southern Canada specifically, we characterized the spatial and seasonal variability in their air and water concentrations using PAS and PWS networks installed across the coastal regions of Quebec (QC) and British Columbia (BC), Canada, which indicated higher levels in populated, urban areas, particularly during warmer deployment periods, and explored their potential for atmospheric deposition using deposition sampling. As one of the banned traditional BFRs, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) served as a comparison to TBEC, which had comparable distribution and levels to that of TBEC. Seasonal trends in atmospheric TBEC and BDE-47 were also explored in urban (Toronto, Ontario) and remote regions (Saturna Island, BC and Tadoussac, QC) using active air sampling (AAS). TBEC and BDE-47 levels correlated with temperature in Toronto and Tadoussac; however, the relationship was less pronounced on Saturna Island. Air mass origin calculations with FLEXPART estimating the impact of anthropogenic emissions on the measured levels of BFRs in this remote region neither fully explain their seasonal variability, suggesting that the atmospheric fate of BFRs on Saturna Island may be subjected to complex, competing influences of air mass origin and temperature. Despite there being no evidence that TBEC has been produced, or imported for use, in Canada, it is now similarly abundant in the Canadian atmosphere as BDE-47. The recorded spatial and temporal variability of TBEC air concentrations suggest that its emissions are not occurring at specific locations but are generally tied to the presence of humans. The most likely explanation for its environmental occurrence in Canada is therefore the release from imported consumer products containing TBEC.

### 3.22.B.T-04 Assessing Chemical Mixtures in Urban Air Using PUF-disk Passive Air Samplers: What Can be Achieved via High-resolution Mass Spectrometry Based Nontargeted Analysis

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Urban air contains complex chemical mixtures from various chemical sources. Chemicals emitted to urban air undergo complex transformations, which act as secondary sources of many chemicals in air. To improve understanding and managing the ever-expanding list of persistent organic pollutants, atmospheric monitoring using passive air samplers provides a cost-effective mean with capability of characterizing environmental exposures of chemicals in air and tracing source attributions. A wide range of chemicals are present in air and sampled by passive air samplers. Evidence has suggested there is a need to capture lesser-known chemicals in air to assess the risk of chemical mixture in air. Gas- and liquid chronography coupled with high-resolution full



spectra mass spectrometer provide a good opportunity to analyze the complex chemicals mixtures in air. The approach referred to as nontargeted analysis (NTA). In this study, we conducted NTA for passive air samplers that were deployed to capture various urban source sectors and based on the variation in concentrations in different groups of samples, we filtered chemicals contributed by specific source sectors (e.g., road) and spent efforts on identifying abundant lesser-known chemical present in urban air. NTA was conducted for 30 extracts of passive air samplers deployed in Toronto to capture influence of various source sectors including traffic, urban, residential, and industrial contributions. Partial least square discriminant analysis (PLS-DA) was used to filter compounds associated with a sample group. A total of 16000 chemical features were extracted from the data. Of the chemical features, 3200 are aromatic compounds with RDBE>8. Chemical features contributing to distinguish the traffic influenced with other sites include some well-known compounds known as from vehicle engine emissions. For example, polycyclic aromatic hydrocarbons such as fluorene, anthracene, etc., and other polycyclic aromatic compounds containing nitrogen and alkyl groups were also found in this cluster of chemicals. For example, 4,5-dimethyl-carbazole, and di-t-octyl-diphenylamine. Di-t-octyl-diphenylamine is known as used in lubricant oil so it can be originated from non-exhaust vehicle emissions. Besides the steric hindered phenylamines, we also observed a number of compounds containing N and O (e.g. C<sub>19</sub>H<sub>23</sub>NO and C<sub>21</sub>H<sub>27</sub>NO). These compounds can be originated from atmospheric oxidation of the precursor compounds.

### 3.22.P-Mo240 Integrated Treatment of Per- and Polyfluoroalkyl Substances in Existing Water Treatment Plants – Scoping the Potential of Foam Partitioning

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Per- and polyfluoroalkyl substances (PFAS) are environmental pollutants known for their persistent, bioaccumulative and toxic properties. They are ubiquitous in the aqueous environment, but existing water treatment technologies are energetically and financially costly. Recently, foam fractionation has been established as a relatively inexpensive and green water treatment method capable of achieving competitive PFAS removal. It works by introducing air bubbles at the bottom of a water column, to which the PFAS molecules adsorb. If surfactant concentrations in the water are high enough, a PFAS-enriched foam can subsequently be separated from the liquid phase, resulting in a relatively PFAS-free effluent.

Many existing water treatment plants use aeration as part of their treatment train. Often, the presence of surfactants or filamentous bacteria may result in formation of foam on top of such treatment processes. We investigated whether foam formation could be exploited for the integrated removal of PFAS within existing treatment processes. Influent, effluent, and foam samples from seven water treatment plants, where foam formation in an aerated process step was observed, were analysed for the concentrations of 29 PFAS. Treatment processes included in the study were activated sludge, moving bed biofilm reactor (MBBR) and electrocoagulation. In addition, correlation analysis of PFAS concentrations with general chemistry parameters were performed. For each treatment plant, the ΣPFAS concentrations in the foam were indeed higher than in the influent, with ΣPFAS enrichment factors ranging between 1.2 and 150,000. Enrichment in the foam was generally higher for long-chain PFAS than for short-chain PFAS. The enrichment was highest in foam from the MBBR processes. Despite the high PFAS concentrations in the foam, no decrease in effluent PFAS concentrations occurred for most plants, indicating that enrichment in the foam does not necessarily lead to PFAS removal. Nevertheless, the high foam concentrations could possibly be used to contrive an integrated PFAS removal by optimizing existing systems. The findings of this novel green treatment technology will be of interest to water treatment plant operators who are trying to decrease aqueous PFAS contamination, but also to scientists or regulators who must consider foam partitioning in their sampling strategies for PFAS.

### 3.22.P-Mo241 Evaluation of Anaerobic Digestion and Diverse Thermal Treatments for the Removal of Organophosphate Flame Retardants (OPFRs) from Norwegian Sewage Sludge

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Organophosphate flame retardants (OPFRs) are chemical additives widely used in combustible materials to prevent fire or delay combustion processes. Due to their properties, these substances are commonly used as plastic and flame-retardant additives in furniture, upholstery, electronics, and paints, among others. As these substances are not chemically bound to materials, they are easily released into the environment through volatilization, abrasion, or dissolution. Their main routes of entry into the environment are through product life cycles, the discharges from wastewater treatment plants (WWTPs) and atmospheric emissions. Particularly in the case of WWTPs, some OPFRs display limited biodegradation during the treatment and high accumulation in sewage sludge, demonstrating that primary and secondary treatments applied in the WWTPs are not sufficient to remove them from the water or sludge. An alternative waste handling technique for sludge is combustion or pyrolysis, which consists of heating up the sludge in the presence or absence of oxygen, respectively. The combustion process produces mainly low carbon ashes, while the pyrolysis process can result in the production of a porous carbon-rich product called biochar that can be used further as soil amendment. Recently, pyrolysis of sewage-sludge has been widely studied as a promising new treatment alternative that promotes circular economy in waste management.

The behaviour of OPFRs during treatment in WWTPs was assessed, as well as the removal efficiencies of these substances during anaerobic digestion and thermal treatments such as combustion and dry pyrolysis. To that aim, raw-, digested-, combusted and pyrolyzed sludge samples collected from different WWTPs located in Norway were analysed. Obtained results demonstrated the presence of sixteen out of 21 OPFRs in digested sludge samples in concentrations up to 2186 ng g<sup>-1</sup> (dry wt; total concentration). Combustion of digested sludge was carried out at 100 and 300°C. The ΣOPFR decreased only 4% at 100°C but reached up to

98% when the temperature was increased to 300°C. As for pyrolysis, the digested dry sludge was subjected to pyrolysis at different temperatures, from 500 to 800°C, and at a residence time of 20 to 40 min. According to the results, pyrolysis was considered sufficient to reduce the concentrations of ΣOPFRs to <10% at the lowest temperature (500°C).

### 3.22.P-Mo242 How Do Trees Capture PAHs? Tree Height Matters and So Does Leaf Shading

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Trees can improve local air quality by capturing **polycyclic aromatic hydrocarbons (PAHs)**, which is especially important in cities with heavy motor traffic, but there are many unknowns about the uptake mechanisms. This lack of knowledge limits the application of models and complicates decision-making when deciding the type of trees to plant in urban environments. We investigated intracanalopy ability of sequestering PAHs along a city road, and some of the factors responsible for the uptake, as well as we proposed hints for selecting best species to enhance capture ability. For this purpose, we selected 15 ornamental apple trees (*Malus × moerlandsii* "Profusion") from a main street in Coruña (Galicia, NW Spain), with a high traffic intensity (average of 12,262 vehicles/day). In each of these trees we sampled leaves from 12 positions corresponding to three variables (3 heights, 2 distances to the trunk, and 2 orientations) and determined the concentration of 16 PAHs and the SLA in each sample. After statistical analysis, we found significant differences between positions, able to explain approximately 30% of the observed variability. The main differences were between heights and, in some cases, between distances to the trunk, with lower and inner leaves having a significantly higher PAH uptake capacity than upper and outer leaves. To determine the importance of SLA in the observed differences, the analyses were repeated with the data corrected for SLA, i.e., expressing concentrations in terms of surface area instead of mass. As a result, we found that the differences between distances to the trunk disappeared and that the  $R^2$  decreased when significant differences were maintained. That is, the concentration differences between inner and outer leaves and part of the differences between leaves of different heights can be explained by differences in SLA. All these results suggest that intra-individual variability is not negligible and should be taken into account in sampling and models designs. Two of the main sources of variability are height (lower leaves uptake more PAHs than higher leaves) and SLA (the higher the SLA, the higher the concentration of PAHs per leaf gram). This may be due to distances to the emitting source, the barrier effect of leaves and leaf illumination (both for its influence on SLA and PAH volatilisation-deposition). Therefore, the ideal vegetation for removing PAHs from the air would be leafy trees or shrubs, not too tall and shaded.

### 3.22.P POPs and Emerging Pollutants: Environmental Fate, Research, and Monitoring at the Interfaces of Science and Policy & Application of Green Removal Technologies

#### 3.22.P-Mo243 Fractionation and Toxicity Study on Produced Water Discharge

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Produced water (PW) is the water co-produced with oil and gas from oil wells and it represents the largest volume waste stream in oil and gas production operations on most offshore platforms. In 2021 there was an estimated 30 million tons of produced water discharged from the Danish continental shelf alone. Current regulations cover only the dispersed hydrocarbon content by the legal limit of 30 mg/L; this limit does not address the polar species, such as naphthenic acids and alkyl phenols dissolved in the water, which are potentially more environmentally concerning constituents in produced water. Produced water is high in content of BTEX (benzene, ethylbenzene, toluene, xylene) together with naphthalene and alkylated naphthalene compounds, naphthenic acids (NAs) and Alkyl phenols (APs). NAs and BTEX come from natural contact of crude oil with water, NAs and APs come from added production chemicals but also naturally occurring in crude oil. Nonylphenols (NPs) and Octylphenols (OPs), are covered by EU regulations and included in the list of priority hazardous substances for surface waters. For detailed analysis, samples of produced water discharge from the Danish South North Sea oil production wells are purged in charcoal trap for volatiles analysis, and then fractionated by Solid Phase Extraction (SPE) leading to the isolation of APs, NAs and other organics. The individual extracts are measured for target analysis with GC-MS and LC-ESI-MS. The ecotoxicity of the extracted fractions is tested using standard toxicology tests on bacteria *Aliivibrio Fischeri* and algae *Skeletonema sp.* The results of this study can help to better understand major drivers of toxicity of produced water discharge together helping to develop better technologies for the offshore wastewaters treatments.

#### 3.22.P-Mo244 Factors Influencing Concentrations of Organophosphate Esters in UK Freshwater Sediment

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Because of their extensive application as flame retardants, plasticisers, and anti-forming agents, the environmental presence and toxicity of organophosphate esters (OPEs) has become a subject of serious concern. As wastewater treatment plants (WWTPs) have been suggested as major sources of OPEs to the aquatic environment, this study evaluates the impacts of WWTPs on OPE contamination, as well as spatial and seasonal variations in OPE concentrations in UK riverine and canal sediment, for the first time. Detection frequencies of the target analytes (tris (chloroethyl) phosphate (TCEP), tris (2-chloroisopropyl) phosphate (TCIPP), tris (1,3-dichloro-2 propyl) phosphate (TDCIPP), tri-n-butyl phosphate (TNBP), tris (2-butoxyethyl) phosphate (TBOEP), 2-ethylhexyl diphenyl phosphate (EHDPP), tris (phenyl) phosphate (TPHP), and tri-m-tolyl phosphate (TmTP)) ranged from 58% to 100%. The relative abundance of Cl-OPEs was TCIPP>TCEP>TDCIPP and the concentration of Σ<sub>8</sub>OPEs ranged

from 107 ng g<sup>-1</sup> (dry wt; in the Worcester-Birmingham canal) to 52 ng g<sup>-1</sup> (dry wt; in both rivers Severn and Sowe). Significantly higher concentrations were observed downstream than upstream of the WWTP for some target OPEs and the highest  $\Sigma_8$ OPE concentration for all study locations was recorded within Autumn months (September, October, and November). Concentrations of  $\Sigma_8$ OPEs in sediment samples from the River Severn, River Tame, and River Sowe all varied inversely with the river level and flow rate. The potential risk posed by the target OPEs in all study locations was found to be low, except for a moderate risk identified for EHDPP in the Worcester - Birmingham canal, while the average concentrations of each target OPE in each study location were lower than those reported in some previous studies but fall broadly in the middle of the range reported from other countries.

### 3.22.P-Mo246 Occurrence of Emerging Contaminants and Microplastics in Distinct River Basins (Urbanized and Rural) in the State of São Paulo, Brazil

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Different land uses and occupations impact directly water quality due to the release of chemicals by anthropic activities. Agricultural areas are associated with pesticides, while urban and industrial areas are associated with personal care products (PCPs), industrial-origin substances, hormones, plastics, and microplastics (MPs). All these different substances are defined as emerging contaminants (ECs). To date, few studies have established a relationship between land use and occupation and the occurrence of these contaminants in surface water. We aimed to assess the impact of different land uses and occupations on the distribution of ECs in surface water in the state of São Paulo, Brazil. Two distinct watersheds were chosen: the Atibaia river basin, located in an urbanized and industrial area, and the Turvo/Grande river basin, located in an agricultural area with a predominance of sugarcane crops. Seasonal samples were collected at three points from each river to analyze the occurrence of ECs and microplastics. The occurrence of 28 ECs of industrial, urban, and agricultural origin was assessed. The contaminants in water samples were extracted by SPE and quantified by LC-MS/MS. Analysis of similarities (ANOSIM) and similarity percentages (SIMPER) were applied to compare the two different watersheds. MPs were collected in each river with a plankton net. The samples were digested and separated by density before characterization by ATR- $\mu$ FTIR. ANOSIM analysis of emerging contaminants showed statistical differences between the watersheds ( $p < 0.05$ ). In SIMPER analysis, 5 out of 28 ECs (caffeine, carbendazim, tebuthiuron, atrazine and ametryn) contributed to 84% of the difference between the watersheds. Caffeine concentrations were higher in the Atibaia river, while the concentrations of the pesticides carbendazim, tebuthiuron, atrazine, and ametryn were higher in the Turvo/Grande river. The most common types of MPs found in this study were white fragments, ranging from 100 to 250  $\mu$ m in size, made of polypropylene or polyethylene. Fibers and different color plastics were found near urban areas, indicating the presence of anthropogenic sources. However, more seasonal sampling is needed to confirm this relationship. Our results showed that the occurrence and concentrations of ECs depend on the use and occupation of the basin since higher concentrations of pesticides were found near agricultural areas, while caffeine concentrations were higher near urban areas.

### 3.22.P-Mo247 Sources of Organophosphate Esters to Coastal Regions in Southern Canada

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To identify and quantify the sources of organophosphate esters (OPEs) to the atmosphere and waters of the St. Lawrence estuary in Quebec (QC) and the Southern Salish Sea region of British Columbia (BC) in Canada, this study relied on two extended passive sampling networks. Overall, more than 150 XAD resin-based passive air samplers and more than 50 polyethylene sheet-based passive water samplers were deployed between November 2019 and April 2022. In both coastal areas, active air and precipitation samples were also collected for a full year at remote sites in Tadoussac, QC, and on Saturna Island, BC. Six OPEs (tributyl phosphate [TBP], tris(2chloroethyl) phosphate [TCEP], tris (1-chloro-2-propyl) phosphate [TCPP], tris (phenyl)phosphate [TPhP]), and 2ethylhexyldiphenyl phosphate (EHDPP) were reliably detected. The average atmospheric concentration levels of these OPEs are similar in the two sampling regions and comparable with values reported earlier. The concentrations of OPEs in air are correlated with the population density around a sampling site, and, in particular, are elevated in densely populated areas such as Vancouver, Montreal, and Quebec City. Changes in the relative abundance of OPEs with distances from such population centres can shed light on the relative atmospheric dispersion potential of different OPEs. Factors influencing the variability in the concentrations in air and precipitation between different seasons are investigated with the help of relationships with ambient temperature and air mass history. Concentrations of the OPEs in water are also related to human activities. For example, in BC, higher TCPP levels in Esquimalt Harbour may indicate the presence of local sources.

### 3.22.P-Mo248 Calibrating a Passive Air Sampler for Polychlorinated Biphenyls: The Dependence of the Sampling Rate on Temperature and the Number and Position of Chlorines

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Confident use of a passive air sampler (PAS) requires knowledge of its uptake kinetics in their dependence on meteorological conditions and chemical properties. Such knowledge is gained through calibration studies. Whereas the XAD-PAS has previously been calibrated for the polychlorinated biphenyls (PCBs), only a limited number of congeners had been quantified. With the increasing interest in investigating the full suite of PCBs congeners, there is a need to understand how the XAD-PAS's sampling rate (SR) may vary with a congener's chlorine substitution. We therefore determined the SRs of a wide range of PCB congeners in the XAD-PAS during a calibration study in Toronto, Canada, involving continuous high-resolution active air sampling and twelve deployment periods ranging from 4 weeks to 48 weeks. Because of the wide seasonal range of temperatures in Toronto, the study further allowed us to investigate the influence of temperature on the SRs of PCBs. Due to the relatively high uptake capacity of XAD resin, the amount of all PCBs taken up in the XAD-PAS increased with deployment length throughout the entire year-long study. This was true even for the most volatile congeners, such as PCB-1. This linear uptake confirms the suitability of the XAD-PAS for quantitative sampling of the full range of PCBs, even for deployment lengths as long as one year. We successfully determined SRs for 66 PCB congeners, including the first-ever reported SRs for 56 of them. The SRs tend to increase with increasing congener volatility. Specifically, the SRs decrease with an increasing number of chlorine substitutions. Within a homologue, the SR is positively related to the number of ortho-chlorines, which is again explained by the effect of ortho-substitution on volatility. The logarithm of the hexadecane-air partition coefficient at 25°C,  $L$ , explains 75% of the variability in the measured SRs of the PCB congeners. The SRs of PCB congeners not detected in this study can therefore be estimated from  $L$ . We also observed slightly higher SRs during warmer seasons. Because volatility increases with temperature, this influence of temperature is consistent with the finding of higher SRs for more volatile congeners. The observed dependence of the SRs of the PCBs on volatility and temperature is consistent with the behaviour expected for chemicals that sorb strongly to XAD-resin from the gas phase and experience a relatively slow sorption kinetics.

### 3.22.P-Mo249 Foam Fractionation for Removal of PFAS from Contaminated Water – Towards Closing the Mass Balance

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Per- and polyfluoroalkyl substances (PFAS) are known for their persistent, bioaccumulative and toxic properties and are notoriously difficult to remove from water. Foam fractionation has recently attracted attention as a low-cost and environmentally green treatment technology for PFAS-contaminated water. The technology works by adsorbing PFAS on rising air bubbles and subsequently sequestering a concentrated foam from the top of the water column, resulting in a PFAS-depleted effluent. However, since foam concentrations are often not determined, data on the mass balance over the foam fractionation process are scarce. Moreover, when foam concentration data are available, gaps in the mass balances are often found. This study verified the high PFAS removal efficiency of a pilot-scale foam fractionation process for treatment of industrial water contaminated with aqueous film-forming foam. ΣPFAS removal reached up to 84 % and the removal of perfluorooctane sulfonic acid (PFOS) up to 97%. Fluctuations in treatment efficiency due to variations in the total organic carbon concentration, pH and turbidity of the influent water were minor, confirming the robustness of the technology. Significant positive correlations between PFAS removal and conductivity as well as influent dissolved salt concentration were observed. Over all experiments, the mass balance closure did not differ significantly from 100%, which was partly due to high measurement uncertainties. Nonetheless, there was measurable PFAS sorption to the walls of the reactor, and high PFAS emissions exited the reactor with the outgoing air. Mass balance closure correlated positively with PFAS emissions in aerosols, which could possibly be caused by underlying correlations with turbidity. The elevated PFAS concentrations in the air around the pilot-scale process have human and environmental health implications for worker exposure and prevention of PFAS releases to the atmosphere, and demonstrate that it is crucial to vent foam fractionation systems to the outside and install appropriate filters on their air outlets.

### 3.22.P-Mo250 Effect of Clarithromycin Addition on Soil Microorganisms of Agricultural Acid Soils

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The presence of emergent contaminants such as antibiotics of human use can provoke important damage on non-target-microorganisms of agricultural soils. The microbial community's response to the clarithromycin addition at seven different concentrations was studied in four acid soils located in Galicia (NW Spain) with different organic C content and similar pH after 8 and 42 incubation days. The phospholipid fatty acid analysis was used to estimate the total microbial biomass and specific fungal, bacterial, actinobacterial, Gram-negative bacterial, and Gram-positive bacterial biomass as well as the microbial community structure (PLFA). The microbial biomass (total and specific groups) was different in four studied soils, the highest values being exhibited by soils with the highest organic C. The antibiotic addition showed a positive effect on microbial biomass (total and specific groups), especially at the highest dose; the effect being more accentuated with time passed after the addition (42 days > 8 days). Principal component analysis (PCA) of the PLFA data performed with the whole data set showed that the main determining factors of the microbial structure followed the order: soil > time incubation > antibiotic dose. When the PCA was performed separately for each incubation time, the results showed that microbial communities of four soils were different. Likewise, for each soil, different microbial communities were observed depending on antibiotic concentration.

### 3.22.P-Mo251 Clarithromycin Sorption by Different Forest and Crop Soils

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Persistent organic pollutants (POPs) bioaccumulate in the environment being both an environmental and a health issue. Among these compounds, pharmaceutical products, such as antibiotics, represent an important percentage of them, since their use is generalized and increases yearly. Clarithromycin is a macrolide antibiotic used to treat several bacterial infections. It has been detected in the sewage systems of different cities around the globe, which involves the risk of ending up in soils when sewage sludges are used as fertilizers. It is important to determine the sorption capacity of soils to estimate the risk of leaching to groundwater and water courses on one side, or the persistence on the soil and consequently availability for transfer to crops on the other side. The aim of this work was to quantify the sorption capacity for the antibiotic clarithromycin by 21 different forest and crop soils covering a wide range of pH and organic matter content. Batch experiments were performed with 3 forest soils (under pine, oak, and eucalyptus), 6 soils under corn and 12 vineyard soils. Clarithromycin was added at different concentrations (2.5, 5, 10, 20, 30, 40, 50  $\mu\text{mol L}^{-1}$ ) to the soil samples and the adsorbed and desorbed antibiotic concentration was measured using HPLC equipment. The results showed that the forest soils adsorbed less than the crop soils, both under corn and vineyard, especially at the highest antibiotic concentrations added. The maximum value of adsorbed clarithromycin on the forest soils was 77% for the lower antibiotic concentration on the pine soils, meanwhile several of the crop soils adsorbed 100% of the antibiotic added even at the highest concentrations. Regarding desorption, on the forest soils there were not desorbed clarithromycin, which means that the adsorption process is practically irreversible. On the other hand, some of the crop soils presented desorption, one of the corn soils with maximum desorption of 7%, and three of the vineyard soils with high percentage of desorption (50–80%) for the lower antibiotic concentration added, even though for most of the crop soils the desorption was very low or below the detection level. In general, clarithromycin was adsorbed in high quantities by the crop soils, decreasing the risk of leaching and incorporation into the food chain.

### 3.22.P-Mo252 The Impact of Sediment Turbidity on PAH Resuspension and Potential Baseline Toxicity

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Sediments store a wide range of contaminants, particularly hydrophobic ones, such as PAHs. Under high turbulence, sediment particles are resuspended in the water column, where chemicals are released. In the water phase, the freely dissolved concentration ( $C_{\text{free}}$ ) represents the bioavailable fraction of the chemical. This study aims to understand better how sediment resuspension affects PAH leakage from sediment to the water and how this leakage is modulated by PAH hydrophobicity. An experiment was carried out with cores of spiked sediment with 4 PAHs (acenaphthene, fluorene, phenanthrene and fluoranthene; 3  $\text{mg kg}^{-1}$ ) with a gradient in log Kow from 3.9 to 5.2. A polyethylene passive sampler (PE) was added to each core, and different flow velocities were applied to create different resuspension levels. The turbidity was measured and used as a proxy for sediment resuspension. PEs were collected after 2, 3, and 4 weeks of exposure and PAHs were extracted from the PE and quantified by GC-MS. The chemical activity (the effective concentration of a chemical in a compartment, e.g., water) was calculated for each PAH and summed to estimate the baseline toxicity of the mixture.

The turbidity increased with the flow velocity but decreased after two weeks of exposure, most probably due to particle aggregation. The uptake of PAHs by the PE was affected by turbidity and the molecule's hydrophobicity, with a significant interaction between the two variables. At low turbidities ( $<3$  NTU), PAHs were accumulated according to their gradient of hydrophobicity with higher accumulation for lower Kow. However, as turbidity increased, the uptake of congeners with different Kow converged to become similar at  $>7$  NTU. These dynamics are most likely driven by PAH diffusion and desorption from the resuspended particles at low and high turbidities, respectively. Finally, a model ensemble was established to (1) predict  $C_{\text{free}}$  using turbidity and logKOW values from the experiment and (2) estimate the potential baseline toxicity of the mixture from the  $C_{\text{free}}$  values.

Our findings show that using passive samplers in field surveys should be combined with turbidity measurements to obtain PAH concentrations (and, perhaps, other hydrophobic contaminants) under environmentally relevant conditions. Thus, this study contributes to improved hazard assessment of pollutants in the sediments and demonstrates the need to account for the natural variability of resuspension in the environment.

### 3.22.P-Mo253 Efficient Removal of Flame Retardants by Metal Ferrites Nanoparticle Incorporated in Guar Gum Moiety: Green Synthesis and Photoactivity

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In the COVID-19 pandemic, several reports concluded that improper disposal of plastic has caused adverse impact on environment in form of debris and leaching of chemicals present in it. Brominated Flame Retardants (BFRs) mainly Terabromobisphenol-A (TBBPA) used in many plastic industries proved to be endocrine disruptor, persistent and create genotoxicity. Seeing environment safety, the present study investigated the removal of TBBPA from water by using green synthesized ferrites nanomaterials. Seed extract of *Sapindus mukorossi* was used as capping and reducing agent. For structural and morphological analysis, PXRD and different microscopic techniques were used, respectively. The water samples were collected from different fields where plastic wastes were collected and centrifuged to remove solid impurities. The traces of TBBPA were confirmed by LC-MS and UV-Visible spectrometer. Various parameters were also optimized to get maximum removal of TBBPA under sunlight. Green nanomaterial followed both adsorption as well as photocatalytic degradation to convert complex structure of TBBPA into safer metabolites. LC-MS technique was used to find out formation of metabolites formed during photocatalytic degradation. Thus, such metal ferrite photocatalyst could be used as a promising material for application in industrial wastewater treatment processes.

### 3.22.P-Mo254 Organoclay for Sorption of PFAS Mixtures from Natural Waters

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As a class of well-known pollutants, per- and polyfluoroalkyl substances (PFAS) are extremely persistent and potentially bioaccumulative as well as toxic, and have been detected ubiquitously in the environment. In the context of increasing concentrations in the environment over the years, as well as current and new legislations with strict guideline values, the need for cost-effective and environmentally friendly treatment techniques is given. In particular, this involves the removal of PFAS from various water matrices using sorption filter materials.

Organically modified clay materials (Organoclays) have been developed that meet the requirements for an efficient removal option for PFAS. The material is based on low-cost as well as globally available clay and is modified with a sustainable surfactant. In this study, three different organoclays were tested in batch mode for removal of a wide range of different PFAS in aqueous solutions. In addition to the synthesis and characterization of organoclays in terms of active surface area by BET surface area analysis, infrared spectroscopy and thermogravimetric analysis, adsorption experiments have been performed.

At the beginning, the optimal solid/liquid ratio was determined. Experiments were performed as a function of time to determine kinetic parameters. Furthermore, results of PFAS removal from sampled water samples were evaluated. For this purpose, four natural water samples were spiked with a PFAS mixture and the removal was subsequently determined. Comparatively, activated carbon was tested for the same experimental parameters. The results showed higher removal rates with even over 80% removal of short chain compounds such as perfluorobutanoic acid and perfluoropentanoic acid for one organoclay compared to activated carbon. This value is reached after two hours of adsorption. Furthermore, the results from real water samples demonstrated that PFAS removal is lower compared to ultrapure water.

This research highlights that low-cost and sustainable adsorbents for PFAS removal are feasible. Moreover, they can reach similar or even better efficiencies in removing PFAS compared to activated carbon.

### 3.22.P-Mo255 Synthesis of Magnetic $\alpha$ -NiMoO<sub>4</sub>/ZnFe<sub>2</sub>O<sub>4</sub>/biochar and Study of Ketoprofen Decomposition Through Photocatalytic Reaction

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Pharmaceutical organic pollutants have been found in various environments such as surface water, wastewater, and groundwater over the past decades, and these pharmaceutical pollutant residues have a problem that negatively affects aquatic ecosystems and human health. In this study, a heterojunction structure/biochar composite ( $\alpha$ -NiMoO<sub>4</sub>/ZnFe<sub>2</sub>O<sub>4</sub>/ coffee biochar) containing nickel molybdenum oxide and zinc iron oxide capable of magnetic separation was synthesized through hydrothermal synthesis to decompose ketoprofen, one of the pharmaceutical substances. Through a batch reaction, it was confirmed that more than 98% of ketoprofen could be removed within 180 min under irradiation of visible light, and through a reusability test, it was confirmed that there was high decomposition efficiency and chemical stability even after reuse 5 times. In addition,  $\alpha$ -NiMoO<sub>4</sub>/ZnFe<sub>2</sub>O<sub>4</sub>/coffee biochar showed excellent photocatalytic efficiency in the presence of various anions and a wide pH range, proving that it is a promising material for treating water environments. ESR analysis and scavenger test results showed that the hydroxyl radical formed through the photocatalytic reaction plays a key role in the decomposition of ketoprofen. The results of liquid chromatography-tandem mass spectrometry analysis revealed 24 degraded intermediates byproducts indicating three degradation pathways of ketoprofen in this study.

### 3.22.P-Mo256 Screening Chemicals for POP-Like Long Range Transport Behaviour

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Persistent organic pollutants (POPs) are likely as a result of their long-range environmental transport (LRT) to lead to significant adverse human health and/or environmental effects in remote regions. It is therefore of considerable interest to identify organic chemicals with a potential to result in environmental and human exposure in remote regions. However, most simple models motivated by regulatory needs, such as The OECD Pov and LRT screening Tool (Wegmann et al. 2009), do not explicitly calculate the potential for accumulation, and therefore for resulting in exposure, in a remote region. Currently, The Tool calculates (i) the distance it takes to reduce the chemical concentration by 63% in a plug-flow system (CTD), (ii) a transfer efficiency (TE) expressed as the mass flux from the atmosphere to remote surface compartments divided by the emission mass flux in a source region, and (iii) an overall persistence (Pov). Recently, we have developed three new LRT potential metrics, collectively referred to as the emissions fractions approach. Each of these metrics is expressed as a fraction of the emissions in a source region and quantifies the extent to which a chemical has the potential to (i) reach a remote region in both air and water (dispersion,  $\phi_1$ ), (ii) be transferred to surface media in the remote region (transfer,  $\phi_2$ ), and (iii) accumulate in these surface media (accumulation,  $\phi_3$ ). The new metrics were implemented into The Tool's code along with the CTD and the TE. This amended model was used to categorize 12,615 high-production volume chemicals (HPVs) for potential POP-like behaviour using threshold values for  $\phi_1$ ,  $\phi_2$ , and  $\phi_3$  that were derived using a selection of chemicals categorized as POPs. For comparison we similarly categorized the HPVs based on the existing metrics. An assessment based on CTD and TE, unlike one based on  $\phi_1$  and  $\phi_2$ , fails to recognize the potential for LRT in water for numerous chemicals. Even when combined with Pov, the existing LRT metrics therefore cannot fully account for a chemical's potential for accumulation in the remote region as a result of LRT in the same way that  $\phi_3$  does. An assessment based on  $\phi_3$  flags about twice as many HPVs as having POP-like LRT than an assessment

based on the Pov and CTD. The main causes for the discrepancies in HPV categorization, along with the implications for ranking chemicals according to LRT potential, will be highlighted.

### 3.22.P-Mo257 Progressing Modeling of PFAS Bioavailability to Support Water Permitting and Effluent Monitoring Regulations

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The unique chemical properties of per- and poly-fluoroalkyl substances (PFASs) present a challenge for developing regulatory guidelines to protect organisms in the environment. While long-chain perfluorinated alkyl acids (PFAAs) are bioaccumulative and frequently detected in biota and humans, their bioavailability and biological uptake are more complicated than other hydrophobic contaminants, such as PAHs or PCBs. Where PAHs and PCBs bind predominantly to storage lipids, PFAAs have the potential to interact strongly with proteins such as serum albumin, fatty acid binding proteins, and phospholipid membranes, and storage lipids. This challenge is further compounded by the fact that only a fraction of the PFAS structures identified or inferred in environmental samples can be reliably quantified with standard analytical methods. As such our understanding of PFAS bioavailability and environmental risk is incomplete and based on experiments and modelling from a limited domain of PFAS chemistry.

This poster outlines the framework for developing a reliable methodology for estimating biological uptake of PFAS based on protein-water ( $K_{PW}$ ), membrane-water ( $K_{MW}$ ), and octanol-water ( $K_{OW}$ ) partition coefficients. These enhanced methods can better estimate PFAS bioavailability and provide practical guidance for regulatory monitoring and permitting of PFAS while minimizing vertebrate and in vivo testing.

Traditional bioaccumulation models, which solely rely on partitioning to storage lipids and are based on  $K_{OW}$ , do not accurately predict observed PFAA bioaccumulation. Such models do not consider other biologically relevant partitioning of PFAS. Therefore, there is a need to develop novel bioaccumulation models, or incorporate better predictors of biological partitioning, to develop PFAS regulations protective of the environment. These models may include  $K_{OW}$ , as well as  $K_{PW}$  and  $K_{MW}$  partition coefficients.

There is ongoing research to empirically measure  $K_{PW}$  and  $K_{MW}$  values for target PFAS. One particular tool, biomimetic chromatography, has the ability to predict  $K_{PW}$  or  $K_{MW}$  from the retention time on specialized HPLC columns. When coupled with high-resolution mass spectrometry (HRMS), biomimetic HPLC offers the ability to predict  $K_{PW}$  and  $K_{MW}$  values for suspect or non-target PFAS. Coupled with QSAR, measurements of biologically relevant partition coefficients can provide a more comprehensive understanding of the bioavailability of a larger domain of PFAS chemistries.

### 3.22.P-Mo258 Implementation of PFAS Transport Modelling to the Pesticide Fate Models PEARL and TOXSWA

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Per- and polyfluoroalkyl substances (PFAS) are emerging threats to water and food safety. Contaminated drinking water can represent an important share of the multiple sources of exposure to PFAS, while water treatment technologies are costly to implement. The protection of water resources from PFAS contamination is therefore of utmost importance. In this context, the understanding of PFAS transport in soil, groundwater, surface water, and plants would help decision-makers to evaluate the most efficient policy measures to tackle this threat, for instance in the context of management of PFAS-polluted soils and waters (biosolids, municipal, and industrial wastewaters).

PFAS is a group representing thousands of molecules with a wide variety of physicochemical properties, which means that as a group they may show unusual environmental behavior compared to conventional environmental organic pollutants. Consequently, the modeling of PFAS requires adjustment of existing fate models. The models TOXSWA and PEARL are commonly used by the agrochemical sectors and regulators, and allow for state-of-the-art modelling of pesticides transport. Those two models therefore seem obvious candidates to be extended for PFAS modelling in the context of transfer to groundwater, surface water, and plant intake, in relation to agricultural practices such as sewage sludge use or atmospheric deposition.

In this preliminary study, we will collect environmental behavior for PFAS clusters, including the best conceptual representation of their behavior and the corresponding required parameters to allow the simulation of PFAS within the models PEARL and TOXSWA. We anticipate the following process descriptions to be improved or incorporated in our models: (i) sorption/repulsion of electrostatic compounds to various matrices such as soil, suspended solids, sediment or macrophytes, and the influence of inorganic ions thereon, (ii) macropore flow in soils induced by surfactants and (iii) air-water partitioning. The latter might lead to significant losses by volatilisation of neutral PFAS structures from surface water because of the high persistence of PFAS. We will present the challenges arising from this work as well as the recommended next steps.

### 3.22.P-Mo259 Time Trends of Legacy and Emerging SVOCs in a Kindergarten Undergoing “Green” Retrofitting: Phase 1 – Pre-renovation Conditions

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Older buildings are often associated with a higher load of semi-volatile organic compounds (SVOCs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs), while in newer buildings

plasticizers, organophosphate flame retardants (OPFRs), and other plastic additives typically receive more attention. Green building retrofitting, aimed at reducing energy and water consumption, involves the renewal of insulation and ventilation systems, which can greatly impact chemical levels indoors. We investigate the status of chemical pollution in an older urban kindergarten (built in 1952) before renovation. Indoor air and dust in three kindergarten rooms were collected monthly over one year, as well as outdoor air adjacent to the kindergarten, to establish baseline conditions before renovation. Samples were quantified for a broad set of POPs and emerging chemicals, including PCBs, PBDEs, OPFRs, PFAS, PAHs, and current-use pesticides. Indoor dust concentrations were relatively stable over the year, with greater variations between the rooms, attributed to proximity to building materials and classroom equipment. Indoor air had consistent levels of synthetic chemicals over time, with only PAHs having substantial seasonally-driven variations over the year. Indoor air concentrations were consistently higher than in outdoor air for all legacy and emerging chemicals. This is the first phase of a multi-year study that will track building-associated chemical burdens before, during and after green retrofitting. These data provide insight into the major sources of variability driving children's exposure to POPs and emerging SVOCs in a kindergarten and will allow us to evaluate green retrofitting alters the chemical loads in the indoor environment.

### 3.22.P-Mo260 Evaluation of Best Available Techniques for Toxicity Monitoring of Refinery Effluents

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The Industrial Emissions Directive prescribes that industry emissions should be treated using the Best Available Technology (BAT). The parameters that refineries are obliged to monitor are laid down in a Commission Implementing Decision BAT Conclusions (BATC) document for the mineral oil refining sector (REF). The REF BATC specifies monitoring requirements for wastewater, but without any BAT for toxicity monitoring. Past research has typically focused on identifying, quantifying, and measuring toxicity of individual chemicals. However, Effect-Based Methods (EBMs) have recently emerged as useful bioanalytical monitoring tools that can complement chemical analysis measurements of water/whole effluent quality. EBMs use the responses elicited in bioassays to identify and quantify the toxic effects of chemical families in the sample. This work aimed to identify and evaluate *in vivo* and *in vitro* toxicity test methods in order to shed light upon which tests could be appropriate for toxicity monitoring of refinery effluents, such as may be required in future EU legislation.

A total of 13 *in vivo* and 18 *in vitro* tests were evaluated against specific criteria based on a literature review and survey of commercial laboratories. The criteria included validation maturity, performance, use, conduct of test, result interpretation and possible application limitations. Our potential test battery includes the *umuC*, AhR activation, AREc32 activation, *Allivibrio fischeri* toxicity, algal growth inhibition, *Daphnia* immobilisation, and Fish Embryo Toxicity (FET)/qFET or bivalve embryo development assays. The latter two are considered interchangeable depending on the type of water sample tested and relevance for the receiving water body. The battery covers *in vitro* modes of action (genotoxicity, metabolism and oxidative stress) as well as apical *in vivo* endpoints (cytotoxicity, developmental toxicity, immobilisation and growth inhibition). All tests are commercially available, commonly used for the assessment of environmental water samples, sufficiently validated, standardised to an ISO guideline (or one is currently in preparation) and are expected to be responsive to refinery effluents. Ultimately, the applied battery can consist of one or more of the tests depending on the assessment objective, the protection goal of the monitoring campaign, the type of receiving water, and the activity undertaken (e.g., routine monitoring, full site risk assessment etc.).

### 3.22.V POPs and Emerging Pollutants: Environmental Fate, Research and Monitoring at the Interfaces of Science and Policy & Application of Green Removal Technologies

#### 3.22.V-01 Smallholder Farms Irrigating with Water Potentially Containing Contaminants of Emerging Concern: Possible Effects of Contaminated Water on Soil Quality

**Fanelesibonge P Vilakazi, ARC-SCW, South Africa**

Increasing food demand has increased the pressure on farmers to use different chemical inputs such as pesticides to ensure optimum crop yield. Although such chemicals help against food insecurity, they pose a threat to the environment over time as contaminants of emerging concern (CECs). This study evaluates the impacts CECs present in irrigation water and soils have on beneficial soil microorganisms that promote plant growth. Soil and water samples were collected from the North West province, South Africa from 3 vegetable farms. The farms utilize water from the Hartbeespoort dam for irrigation. Soil samples were collected in replicates from the farms at rhizosphere depths of 0–15 cm. Water samples were collected in 5-liter bottles in replicates. Physicochemical parameters such as pH, electric conductivity (EC), and Total dissolved solids (TDS) were measured using a multiparameter meter. Nutrients were analyzed at the Agricultural Research Council - Soil, Climate and Water (ARC-SCW) analytical laboratories following standard procedures. Furthermore, Soil samples were classified by grain size using the sieve method. Obtained preliminary results were compared against the South African Irrigation Water Standards. The water results varied across the farms with Farm 2 and Farm 3 having a higher pH than the recommended range of 6.5–8.4. Farm 1 had more than double the TDS levels compared to Farm 3 with all EC ranging above the recommended 40 mS/m. All water samples had higher levels of bicarbonate ( $\text{HCO}_3^-$ ) and sulfate ( $\text{SO}_4^{2-}$ ) commonly associated with industrial waste. Only Farm 1 had a pH within the recommended range of 6–7. The soils had a neutral to moderately alkaline pH of 6.6 to 7.8. Farms 1 and 2 were classified as clay soils and Farm 3 was sand-dominated thus sandy loam. As such Farm 1 and 2 had higher nutrient concentrations and calcium (Ca) was the most abundant hence the high soil pH. Higher nutrient concentrations in the soil are beneficial for soil productivity. However, when pH is too high or too low it can affect the soil functions and the availability of nutrients to the crop. Preliminary results suggest that irrigation water quality potentially has a negative impact on soil nutrients and cause changes in



soil microorganisms that affect crop productivity. Results from this study will give insight on how much CECs affect the environment, shed light on the regulation, monitoring and mitigation methods for a sustainable use of pesticides.

### 3.22.V-02 Chlorinated Organophosphate Flame Retardants in Childcare Articles

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Cl-OPFRs are chlorinated organophosphate flame retardants that are added to materials to help meet fire safety regulations. As flame retardants, they are used in many commercial products like childcare and nursery items. The most widely used Cl-OPFRs are tris(1-chloro-2-propyl) phosphate (TCIPP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), and tris(2-chloroethyl) phosphate (TCEP). Concentrations of Cl-OPFRs were measured in waste childcare articles from the Republic of Ireland ( $n = 274$ ) between 2019 and 2020 and from Birmingham, UK ( $n = 89$ ) in 2022. Irish samples ( $n = 274$ ) include fabric and foams from child car seats ( $n = 150$ ), cot mattress ( $n = 21$ ), pushchairs ( $n = 46$ ), pram ( $n = 24$ ), changing mat ( $n = 23$ ), and a miscellaneous of other samples ( $n = 10$ ) from play mats and baby seats. UK samples ( $n = 89$ ) include fabric and foams from childcare articles including baby beds, pushchairs, baby feeding seats, baby walkers, play mats, sleeping bags, child car seats, and child booster seats. Considering the carcinogenic nature of Cl-OPFRs and their adverse effects on children, the European Union has made a proposal to limit their use to 1000 mg/kg by weight in childcare articles. Our results show that out of the 274 Irish samples examined, concentrations of Cl-OPFRs exceeded 1,000 mg/kg in 14 samples (5%) for TCEP, 58 samples (21%) for TDCIPP and 75 samples (27%) for TCIPP. A maximum TCIPP concentration of 170,000 mg/kg was found in cot mattress foam, while TDCIPP and TCEP maximum concentrations in car seats were respectively 390,000 and 66,000 mg/kg. On the other hand, out of 89 UK samples, 3 (3%) and 10 (11%) samples exceeded 1,000 mg/kg for TDCIPP and TCIPP, respectively. The maximum concentration of TCIPP (10,000 mg/kg) was found in changing mat fabric, while TDCIPP maximum value (49,000 mg/kg) was identified in booster seat foam. In addition to the waste management implications of our findings, our data raises concerns regarding occupational exposure in waste facilities to Cl-OPFRs and the exposure of children to Cl-OPFRs during the period of items use.

### 3.23.A Sources, Fate, and Effects of Metals in the Environment

#### 3.23.A.T-01 Metal Analysis in Low-Volume Tissues Using LA-ICP-MS: Benefits for Non-lethal and Non-invasive Biomonitoring

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Conventional analytical techniques for metals often require tissue sample volumes that present challenges and inefficiencies to environmental monitoring programs for industry. Non-lethal sampling of fish is encouraged (e.g., tissue plugs), yet may not meet sample volume requirements for conventional bulk tissue analysis for metals. Enough samples mass is required to meet analytical detection limits, and achieve high accuracy and precision. We highlight a novel method using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to quantify metals in low-volume samples of fish tissue. We conducted an interlaboratory study that demonstrated the method's accuracy, precision, and comparability to conventional bulk analysis using acid-digestion ICP-MS. The objective was to stress-test the ability of various commercial laboratories to meet data quality objectives in the face of sample volume constraints for ovary and dorsal fish muscle tissue. Four laboratories, employing either LA-ICP-MS or acid-digestion ICP-MS, analyzed blind a suite of identical samples and data were compared against *a priori* data quality objectives for accuracy, precision, and sensitivity. Data quality tended to decrease with decreasing sample volume, particularly when samples were less than the minimum volumes requested by the participating laboratories. Effects of sample volume on data quality were not consistent between laboratories or tissue types. For small sample sizes the LA-ICP-MS method was comparable to or slightly better than laboratories employing acid-digestion. These results highlight the potential for LA-ICP-MS to generate high quality data with low sample volume (i.e., 3 mg), and could support non-lethal biomonitoring initiatives.

#### 3.23.A.T-02 Influence of Earthworms on the Bioavailability of Metals and Radionuclides in Soil

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While soil characteristics such as pH, cation exchange capacity, texture and organic matter are known to influence the mobility of pollutants in soil and the transfer to vegetation, far less attention has been paid to the possible non-trophic influence of organisms on pollutant transfer to other species. When pollutants are present in a bound unavailable form in the mineral or organic fraction, they can be made available for uptake by plants through bio-weathering and decomposition of organic material, which is the role of decomposers such as bacteria and earthworms. Within this study, we aim to evaluate the influence of earthworms on the bioavailability of metals and radionuclides in soil.

Belgian soils, historically contaminated with radionuclides and metals, were used. Especially As, Cd, Cu, Ni, Pb, and Zn are present in concentrations above the remediation reference values for natural and agricultural lands in Flanders, Belgium. From a radiological point of view, <sup>226</sup>Ra levels are of most concern. After an 8-weeks incubation period of the soil with or without earthworms, microcosm systems were used to mimic the natural interactions between soil, earthworms, and vegetation. The microcosm system consisted of PVC tubes (diameter 10 cm, height 30 cm) filled with 2.2 kg of wetted soil to which earthworms were added. Reference systems had no earthworms. On each column, 0.6 g ryegrass seeds were sown. After 3-week growth, grass and soil samples were collected and radionuclide and metal concentrations were determined in soil, pore water and grass. In addition, soil pH, dissolved organic matter and the ionic composition of pore water were analyzed.

Results show that earthworm presence increased the concentration of  $^{137}\text{Cs}$ , Cd, Cu, Ni, Pb and Zn in pore water. However, this was not translated into higher uptake levels by grass. Only for Pb, higher concentrations in grass were found. On the other hand, while no difference was observed for As in pore water, higher As levels were present in grass grown on earthworm worked soil. To evaluate possible direct effects of earthworms on pollutant bioavailability, soil pH, the ionic composition of pore water and dissolved organic carbon were determined. Results indicate that earthworms lowered soil pH and increased ion concentrations in pore water.

These results indicate that biologically induced release or remobilization of pollutants in soil should be considered in risk assessment and site management strategies.

### 3.23.A.T-03 Mechanism of Arsenite Immobilisation by Different Zerovalent Iron-based Sorbents in Contaminated Soils

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Zerovalent iron-based sorbents are used frequently to remediate contaminated soils *in-situ*, but their effect on arsenic-contaminated soils is less well known, especially in aquifers where reducing conditions prevail and the dissolved As speciation is dominated by arsenite (As(III)). We compared the kinetics of immobilisation of As by four different zerovalent iron-based sorbents in a contaminated soil where reducing conditions prevail using both batch and column tests in a glove box. XANES analysis showed that solid-phase As in control soils (no sorbents added) was dominated by arsenate (As(V)) even though dissolved As in both the field as well as lab tests was predominantly As(III). As(V) was reduced to As(III) in controls soils after a certain period of time in both batch and column tests suggesting a slow reduction of As(V) controlled dissolved As speciation and concentrations in these soils. The immobilization of As by uncoated zerovalent iron in both micro or nano form was highly efficient and sustainable on the long term reducing As concentration to close to detection limits. XANES analysis showed that both Fe(II) and Fe(III) oxides were formed and it is hypothesized that As was both co-precipitated and adsorbed by these oxides. Sulfidated zerovalent iron products were less efficient, despite previous lab studies showing their efficiency in pure systems. Non-sulfidated zerovalent iron thus appears highly efficient in these lab studies to immobilise As in reducing conditions and these sorbents are now being tested in the field on a pilot scale.

### 3.23.A.T-04 Behaviour of Advanced Materials in Environmental Aquatic Media - Dissolution Kinetic and Dispersion Stability of Metal-containing Perovskites for the Automotive Catalysis Sector

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Industry develops increasingly complex and advanced materials (AdMa) while at the same time incorporating the Safe and Sustainable by Design (SSbD) concept into R&D goals. Perovskite-based three-way catalytic converters are an example of AdMa that may potentially replace some of the currently used benchmarks in the field of automotive catalysis. To function properly, all automotive catalytic converters contain noble and/or heavy metals. The metals of potential concern in these materials are cobalt (Co) and nickel (Ni) which are present along with lanthanum (La) in the catalyst structure. Hence, understanding their fate and behaviour is crucial to assess possible detrimental effects on the environment.

We aim to give insights into perovskite's behaviour and fate in simulant waters, with different ionic strength and salt content, compared to well-known nanomaterials (i.e., ZnO and BaSO<sub>4</sub>), further considering NOM contribution to both dissolution and agglomeration processes. The validity of current OECD guidelines (GD 318 and TG 318) has been tested for AdMa and methodological insights toward their assessment is given. Moreover, evidence on transferability and applicability to other case studies is provided. Our findings rank perovskite's dissolution kinetic in between the two reference materials ZnO and BaSO<sub>4</sub>. The ionic strength of the media does not seem to impact their overall leachable mass % but plays a role in the kinetic of metals release, which was found incongruent and metal dependent. NOM are shown to increase the dissolution of both benchmarks, however no strong effect is observed for the perovskite materials. Dispersion stability of perovskites in solution is instead substantially increased by presence of NOM and decreased by higher Ca content in the buffer.

The presented discussion can raise awareness on the current and future challenges when testing AdMa, highlighting trade-offs in their chemical and physical behaviour, in different simulant waters.

### 3.23.A.T-05 Fish Mercury Relevant to Geographic Variations in Socioeconomic Development

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Fish consumption is a principal route of human exposure to mercury (Hg) and the associated health concern propels global efforts in Hg pollution management. Socioeconomic activities, affecting such factors as emission and aquatic cycling of Hg, fish ecology, and environmental conditions, play a profound role on fish Hg accumulation. In examining the geographic patterns of fish Hg and the possible controlling factors through a comparative study between China and the United States (US), we show that significantly lower fish total Hg (THg), methylmercury (MeHg), and MeHg% are present in China than in the US. Lower Hg levels in baseline organisms, shortened food chain lengths, shorter individual lifespans, and higher individual growth rates contribute to the lower fish Hg in China, despite higher emission, deposition, and environmental concentrations of Hg in China.

We propose a top-down approach to relate geological variability in fish Hg to human activities, where the socioeconomic development status is deemed to determine Hg emission and hydrosphere landscape which in turn affects Hg bioaccumulation through regulating Hg biogeochemistry and food web ecology. This study provides implications on considering regional variability and climate change when managing Hg pollution as a global issue.

### 3.23.B Sources, Fate, and Effects of Metals in the Environment

#### 3.23.B.T-01 Can Plastic Pollution Affect the Environmental Fate of (Trace) Elements? A Preliminary Investigation in Water

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Plastic pollution is a well-known environmental issue, yet several side-effects on the ecosystems are still mostly unknown. A worrying side-effect is the interaction of plastic with (trace) elements, causing potential alterations of their environmental fate. Plastic ageing processes (i.e., plastic alteration in response to physical, chemical and biological agents) seem to play a pivotal role in regulating plastic-(trace) element interaction, but it is still unclear which agents mainly regulate this phenomenon. Therefore, we assessed which ageing factors can affect adsorption properties of plastic toward (trace) elements. Plastic fragments composed by different polymers (polyethylene, polypropylene and polylactic acid) were tested. Three different ageing processes were analyzed: UV ageing (known to induce surface oxidation and change surface charge), biofouling (using different microalgal species) and the sequential application of both processes. Then, adsorption rates and kinetics were tested using aluminium (Al) and copper (Cu) as target elements (at 1mg/l in 0.01 M NaNO<sub>3</sub> buffer solutions). These elements were selected for their role as (micro)nutrients, but potentially toxic if concentrated. Plastic was also characterized considering micromorphology, surface functional groups and water contact angle to observe how surface properties are affected by ageing process. Results highlighted that polymer type limitedly affects adsorption processes of the target elements in water solutions, while UV ageing shows few changes on adsorption rates of plastic, presenting high variance among polymer type and adsorbed element. Biofouling of plastic is instead a key process in regulating this phenomenon: up to 45% of the dissolved Cu and 25% of the dissolved Al are adsorbed by biofilm covered plastic fragments from water solutions. The surface analysis of plastic samples reveals an increase in wettability induced by biofouling, as well as the increase in functional groups typical of peptides (primary and secondary amides) and carbohydrates which are the likely chemical groups enhancing the adsorption capacity of plastic toward metals. The results confirm that environmental ageing of plastic affects the adsorption of (trace) elements and highlight biofouling as the main factor regulating this process. Biofouling, therefore, should be addressed in greater detail to understand the effect of plastic on the fate of (trace) elements in the natural environment.

#### 3.23.B.T-02 Acute and Chronic Effects of Olivine Exposure in the Marine Amphipod *Gammarus locusta*

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Active removal of carbon dioxide (CO<sub>2</sub>) from the atmosphere is needed at a gigaton scale in the next decade to keep global warming below 1.5°C. Coastal enhanced silicate weathering aims to increase the natural process of ocean carbon sequestration via chemical weathering finely ground olivine (Mg<sub>2x</sub>Fe<sub>2(1-x)</sub>SiO<sub>4</sub>) rich rock dispersed in dynamic coastal environments. However, the environmental safety of the technique remains in question since olivine contains relatively high amounts of nickel (Ni) and chromium (Cr) which are released to the environment on weathering. This study investigated the acute and chronic effects of olivine exposure in the benthic marine amphipod *Gammarus locusta*. During a first experiment, Ni and Cr accumulation were determined in adult amphipods exposed to different olivine grains sizes for 24 hours. Acute olivine exposure resulted in significant Ni and Cr accumulation, with higher accumulation observed for smaller olivine grain sizes. Microscopic examination of the guts revealed high amounts of olivine particles, indicating the importance of olivine ingestion as a metal exposure pathway. During a second experiment, juvenile amphipods were exposed to natural sediment mixed with different olivine loadings for 35 days. Amphipod survival, growth, reproduction, metal accumulation and malondialdehyde (MDA) concentrations, as a biomarker for oxidative stress, were assessed at the end of the experiment. The Ni and Cr body burden and MDA concentrations increased with higher olivine exposure concentrations and resulted in a significant reduction of amphipod survival, growth, and reproductive success. A highest no observed effect concentration (NOEC) of 1 w/w% olivine (23 and 62 mg/kg Ni and Cr, respectively) was observed for total juvenile production. This NOEC is low compared to possible coastal olivine application concentrations (0.1 – 8 w/w%) and marine sediment Ni and Cr environmental quality standards (43 and 52 mg/kg, respectively). Overall, our results show that fine grained olivine (< 100 µm grain diameter) can easily be ingested by *Gammarus locusta* and lead to toxic effects during chronic exposure at potential coastal application concentrations.

#### 3.23.B.T-03 Monitoring of Trace Metals in Global Air: Results from the GAPS and GAPS Megacities Networks

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Trace metals are ubiquitous components of various raw materials and industrial products, and enter the atmosphere through anthropogenic activities and the suspension of natural dust. Many trace metals pose risks to both human health and the wellbeing of natural environments. It is essential to monitor trace metal emissions and generate baseline ambient concentrations to guide risk assessment and public policy. Polyurethane foam disk (PUF) passive air samplers (PAS) are a cost-effective, versatile means to monitor trace metals in ambient air. They are capable of capturing both gas- and particle-phase contaminants, and samples therefore represent the entire air mixture. In 2019, 51 Global Atmospheric Passive Sampling (GAPS) and GAPS Megacities Network locations deployed PUF-PAS to assess air concentrations of 25 metal species. PUFs were exposed for periods of 3-12 months, with metal concentrations determined in both acid- and water-soluble fractions. Time-averaged air concentrations were

derived for each metal. Crustal enrichment factors were also determined to evaluate relative anthropogenic impacts and possible source sectors. High crustal enrichment factors were observed for Sn, Sb, Se, Zn, and Mo, which indicates notable anthropogenic inputs of these metals into the atmosphere. Concentrations of Cr, Co, Ni, Cu, As, Ag, Cd, and Pb appear to be the result of mixed crustal and anthropogenic sources. Up to 40% of analyzed metal species were found to be enriched beyond expected crustal concentrations. Total trace metals were highest in Mendoza (Argentina) and New Delhi (India), while lowest total metals were observed across background and polar sites. High proportions of total metal concentrations are attributable to Al and Fe, resulting from soil and dust suspension. Se displayed relatively consistent concentrations globally, suggesting widespread Se contamination. Coal, fossil fuels, and vehicular sources appear to have been influencing factors for a number of trace metals. Al, Cr, Co and Zn were primarily found in the water-soluble fractions, suggesting higher bioaccessibility of these species. Cities with high PM concentrations broadly exhibited higher concentrations of trace metals. Future work will continue to provide cost-effective and time-integrated global baseline and monitoring data for trace metals using PUF-PAS in the GAPS and GAPS Megacities Networks.

### 3.23.B.T-04 Field Application of a Model of Proton and Metal Mixture Bioavailability and Effects

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Quantitatively predicting of the responses of freshwater ecosystems to the effects of potentially toxic metals and acidification is an ongoing challenge in ecotoxicology. The WHAM- $F_{TOX}$  model is based on the assumptions that toxic effects of protons and metal cations are additively related to their occupancies of binding sites on organisms, and that those binding sites can be represented by the binding sites of humic acid (HA). We applied the model to simulate the species richness ( $n_{sp}$ ) of crustacean zooplankton in acid- and metal-contaminated lakes near Sudbury, Ontario between 1973 and 2006. Historic emissions from metal smelters at Sudbury have caused contamination of surrounding lakes by acid deposition, while lakes closest to the smelters were also contaminated with metals, mainly Ni and Cu, and to lesser extents Zn, Cd, and Pb. Changes in water chemistry over the study period show partial recovery as a result of emission reductions.

In application, binding of protons and metals to organisms is simulated by applying the WHAM7 model for each water sample. A combined dose term,  $F_{TOX,i}$ , is computed for each species within a conceptual assemblage, assuming a distribution of species sensitivities to metals. The probability of finding the species in a sample is then computed from a fixed relationship with  $F_{TOX,i}$ , and  $n_{sp}$  is computed by summing these probabilities across all species. The distribution of species sensitivities is found by assuming them to be lognormally distributed and fitting their mean and standard deviation.

The model was able to describe the variability in  $n_{sp}$  well, with an R-squared value of 0.84 ( $p < 0.0001$ ). Generally, the model reproduces the temporal patterns of  $n_{sp}$  in individual lakes well, although it tends to underestimate the rate at which species richness increases as water chemistry recovers. The most important toxic cations were H, Al, Ni, and Cu, with a small contribution from Zn. The predicted contributions of protons and individual metals to toxicity generally showed declines over time in the contributions of protons and Al as recovery from contamination took place. However, in some cases the contribution of Ni increased over time, despite reductions on the dissolved concentration. This illustrates the potentially complex interplay among water chemistry variables determining metal exposure.

### 3.23.B.T-05 Using a Dynamic Energy Budget Model to Investigate Nickel (Ni) Toxicity to *Lymnaea stagnalis*

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*Lymnaea stagnalis* has been shown to be among the most sensitive species for a variety of metals, including nickel (Ni).

However, the observed sensitivity of *L. stagnalis* to nickel is highly variable between studies, without a clear explanation for this variability. The exact mechanism of nickel toxicity to *L. stagnalis* also remains unclear. Under dynamic energy budget (DEB) theory, different physiological modes of action (PMoAs) result in the emergence of distinct changes to the life histories of exposed organisms. DEB models provide a mathematical framework connecting chemical stress at the physiological level to individual-level effects. Consequently, DEB-IBMs can be useful both in determining the PMoA of nickel at an individual level and predicting population level sensitivity.

We calibrated the *Lymnaea stagnalis* DEB-IBM to apical toxicity test data and use the calibrated model to predict nickel toxicity effects on *L. stagnalis* populations. Since no single PMoA emerged as most plausible, several PMoAs were extrapolated to the population level. These population simulations indicated that the relationship between individual sensitivity and population sensitivity is PMoA dependent, further underscoring the need to identify the primary PMoA of nickel to *L. stagnalis*. As a partial validation for our calibrated model, we attempted to predict population level outcomes for *L. stagnalis* as observed in a mesocosm study. In this validation work, simulations assuming increased growth costs as the PMoA consistently provided the best predictions of snail population densities in the mesocosms. However, it should be noted that this validation has important limitations, and the results should be interpreted cautiously. To further examine the PMoA of nickel to *L. stagnalis*, ongoing experimental efforts are investigating the impact of nickel on multiple endpoints, including growth, ingestion, respiration, and reproduction. The experiments have demonstrated a notable difference in the sensitivity of juvenile and adult snails.

### 3.23.P Sources, Fate, and Effects of Metals in the Environment

#### 3.23.P-We194 Metals Availability in Polluted Fibrous Sediments, Relevant Information for Data-Based Environmental Decision-Making

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Before being prohibited in 1969, unregulated wastewater discharges from the Swedish pulp and paper industry created large deposits of polluted fibrous sediments on the Baltic Sea floor and at the bottom of lakes and rivers, throughout Sweden. These anthropogenic, organic-rich sediments are typically heavily polluted by toxic elements (metals and metalloids) and organic pollutants such as PCBs, chlorinated pesticides, or polycyclic aromatic hydrocarbons, known for their toxicity and persistence in the environment. In addition, the degradation of their high content of organic matter under hypoxic conditions leads to the formation of highly toxic methylmercury and the biogenic production of CH<sub>4</sub> and CO<sub>2</sub>, two potent greenhouse gases. It is estimated that approximately 10.8 million tons of fiber material were discharged into the Swedish aquatic environment. Fibrous sediments entail a serious threat to human and ecological welfare, and are of high concern for governmental authorities, researchers, and society in Sweden and other pulping countries such as Finland, Canada, and the USA.

Genuine remediation actions should be taken, considering the best suitable ways to uptake and treat the material with minimal impact on the ecosystem. Additionally, resources could be recovered from the sediments, such as valuable metals or reuse for energy production. In any case, pollutants mobility and/or availability in fibrous sediments, which organic nature differentiate them widely from other polluted sediments, requires further and comprehensive understanding in order to produce a well-founded framework for decision making. The aim of this work, is to study metals speciation, using metals sequencing procedures, in different types of fiber banks and fiber-rich sediments, in a first attempt to understand these processes and provide useful information for their management alternatives. The type and amount of organic matter, the reductive anoxic conditions due to biological degradation under water, the pH and water salinity conditions, among many other factors, will affect to a large extent the distribution of metals in these materials. Thus, this study can aid in predicting metals fate for environmental risk assessments and funded decision making. Further analyses will include the content of organic matter, ligning, and particle size distribution of the material to find possible correlations between these factors and the metals secuenciation in the studied fiber banks.

### **3.23.P-We195 Trace Metals Dynamics in a Loamy Sand Soil after Application of Non-source-separated Biogas Digestate: A Soil Column Study**

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The push for an economic transition towards a circular bioeconomy is constantly growing as global demand for renewable energies and sustainable development increases. Anaerobic digestion (AD) has become a significant element of the circular economy in Europe. One of the AD process's end-products is digestate, which is frequently discarded in landfills because it does not comply with quality criteria imposed by legislations. New strategies for digestate use are needed to improve circular bioeconomy around AD, like its use as amendment for marginal land restoration and bioenergy crop production. However, digestate may contain different types of compounds, as trace metals, resulting in environmental contamination. Therefore, it is important to assess metals dynamics and behaviour in soil systems after digestate application. With this aim, a 90-day mesocosm soil column experiment was conducted to evaluate the fate of trace metals, namely Zn, Cu, Pb, Cr, in a fine loamy sand soil after the application of a layer of digestate-amended soil (14:1, soil to digestate dry wt), with the digestate originating from the organic fraction of municipal solid waste. Two experimental conditions were defined. The first used natural digestate, while the second used digestate spiked with metformin (antidiabetic drug). Soil samples were taken along the columns' lateral sampling ports on days 1, 7, 21, 35, and 90, and were analysed for trace metals' total concentrations and fractionation via atomic absorption spectroscopy. Overall, results showed that metals migrated over time from the top amended soil layer to the underlying one, whereas no migration to the deeper layers was observed. This agreed with trace metals being present in more bioavailable forms in the amended soil layer, and predominantly in immobile less bioavailable forms in the other soil layers. The results also showed that metformin did not significantly influence trace metal behaviour; however, interactions with other potentially present contaminants should be considered. This study shows that digestate can be a valuable resource for marginal land restoration coupled with appropriate bio/phytoremediation technologies that allow for excess pollutant removal from the soil system in situations where more developed technologies/processes are not available or economically feasible.

### **3.23.P-We196 Using a Risk-based Approach to Monitoring of Targeted Sites on Water Bodies in Proximity to Historical and Operational Mine Sites in Ireland**

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Anthropogenic activities such as mining are a source of pollution to the aquatic environment. Mining has been identified as a significant pressure on Ireland's aquatic environment and historical and operational mining activities are point sources of metal contamination in water bodies. Individual water bodies may come under pressure and are at risk of not achieving their Water Framework Directive (WFD) water quality objectives. Previous studies have already reported elevated concentrations of metals – in surface water; stream sediments; mine water discharges and solid mine waste – at historically mined areas throughout Ireland. Using a risk-based approach to monitoring water bodies in proximity to historical mine sites and operational mines in Ireland will better inform WFD surface water monitoring programmes and enable us to perform statistically robust chemical and ecological

status assessments. The Environmental Protection Agency (EPA) investigated 90 river monitoring stations on waterbodies across 22 mine sites in Ireland over a two-year monitoring period (2020–2021). River monitoring stations upstream and downstream of mine sites were selected. Arsenic, cadmium, copper, lead, mercury, nickel, and zinc concentrations in surface water samples were measured using inductively coupled plasma source mass spectrometry (ICP-MS). Supporting analysis of pH, dissolved organic carbon, calculated hardness and calcium content were also carried out as they can influence metal toxicity and are used for bioavailability corrections of lead and nickel concentrations. Metal concentrations were compared with annual average (AA) and maximum allowable concentration (MAC) environmental quality standards (EQS) as set out in Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. The results of this study have been used to inform future WFD Monitoring Programmes and to carry out chemical and ecological status assessments of water bodies in proximity to historical and operational mine sites in Ireland. This study highlights the importance of targeted monitoring and data-driven decision making when designing monitoring programmes for water quality assessments.

### **3.23.P-We197 PhysChemEuro: Introducing a New, Comprehensive Aquatic Chemistry Database of European Waters**

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Bioavailability is a critical concept to understand and evaluate the ecotoxicology of metals. Recently, models have been developed with the ability to incorporate metal bioavailability into the calculation of environmental threshold concentrations (i.e., PNEC, EQS). These models (e.g., Biotic Ligand Model (BLM)) and associated user-friendly platforms (e.g. Biomet), can predict environmental threshold values for several metals including Cu, Co, Ni, Pb, and Zn throughout most European and North American freshwaters. Although substantial progress has been made in the development and validation of these models, their application still depends greatly on the availability of site-specific water quality data for physico-chemical parameters (e.g., hardness, pH, organic carbon). Although some databases already exist (e.g., FOREGS, Waterbase), most resources currently do not contain the range of parameters necessary to perform these analyses, or they fail to report specific details surrounding the geolocation of sampling sites. To address these concerns, the metals research organizations and their partners sought data from European member states and the ECHA Waterbase database to develop a queryable comprehensive database of water quality parameters measured in European waterways. Data were obtained containing records from 1978 to 2022, and the developers intend to perform regular updates to maintain and expand the tool's applicability. Due to the size and scope of this database, a web-based "Shiny application" was developed to enhance user-experience and to assist in the management and query of the database. The resulting web-based tool is publicly available and provides a mechanism to search extant data for water quality parameters filterable by region, country, or location and to obtain simple statistics for each (i.e., mean, percentiles, etc.). It is anticipated that this tool will be valuable in supporting bioavailability calculations and other pertinent analyses related to derivation of environmental thresholds.

### **3.23.P-We198 A Survey of Copper in Sediments and an Assessment of Potential Stressors to Resident Benthic Communities**

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Copper (Cu) is an essential trace element but can be toxic to biota when present above a certain threshold concentration. Cu enters the aquatic environment mostly linked to particulate debris which settles and is absorbed into the sediment. As a result, surface sediments constitute the major store and sink for copper and other pollutants in aquatic ecosystems. The bioavailability of copper in sediment is controlled by multiple factors including Acid-volatile sulphides (AVS). Sediment is likely non-toxic if the concentration of AVS exceeds concentrations of simultaneously extracted metal (SEM). Therefore, accurate and representative measurements of AVS and SEM in sediment are critical for any ecological risk assessment for divalent metals such as copper. This study presents a global survey of AVS and a focused review of copper concentrations in sediments of a well-studied Californian agricultural catchment. Also, a comparison of different metals as potential stressors to benthic communities is presented.

AVS measurements were reported from 21 different countries and ranged from 0.01 to 503  $\mu\text{moles/g}$  for 140 different waterbody types. There was high variability within each waterbody type as well as large variability of AVS within specific locations which were often multiple orders of magnitude different with respect to concentration range.

Total copper sediment concentrations from Cache Slough, ranging from 19 to 63  $\mu\text{g/g}$  dry weight, were similar to concentrations reported in European agricultural streams. Concentrations were not reported to increase over time in this agricultural waterbody where copper was used as a fungicide, although some spatial differences in SEM copper sediment concentrations were reported. A comparative analysis of total copper and SEM copper showed that the range of mean seasonal concentrations of SEM copper was much lower, and more sites showed declining trends for SEM copper than for total copper.

Finally, the importance of individual metals within a metal mixture were assessed as potential stressors to resident benthic communities. Arsenic, lead, and cadmium showed the highest number of statistically significant and ecologically meaningful relationships with benthic metrics. Copper was not reported to show any statistically significant relationships with any of the benthic metrics.

These results provide essential data for assessment of ecological risk of copper to the environment.

### 3.23.P-We199 Development and Evaluation of a Multimedia Model Assessing the Fate, Transport, and Speciation of Heavy Metals in Korea

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Heavy metals (HMs) can exist in multiple species in the environment, and their toxicity varies with species. For more accurate and reasonable risk assessments of HMs in Korea, this study develops a multimedia model that simulates the fate, transport, and speciation of 13 different HMs including Al, Co, Ni, Cu, Zn, Pb, Cr, Mn, Fe, Cu, As, Se, and Hg. In the model, HMs speciation is modeled based on equilibrium and kinetic approaches. Dissolved and particulate phases of HMs are calculated using partition coefficients, which are determined by soil organic matter, pH, and total concentration of HMs. The dissolved phase includes free ion, dissolved organic matter (DOM)-metal complex, and inorganic-metal complex, and V.MINTEQ is used to estimate their distributions. Oxidation-reduction states (for Cr, Cu, Fe, Mn, As, Hg, and Se) and organic-inorganic states (for As, Hg, and Se) are simulated by considering the relationships between redox rates and between methylation/demethylation rates, respectively. The ratios of the predicted to measured (average) total concentration in water (i.e.,  $C_{ratio} = C_{predicted}/C_{measured}$ ) are 0.24 (Al), 0.11 (Cd), 0.28 (Co), 0.29 (Ni), 2.67 (Pb), 0.32 (Zn), 4.20 (Cr), 1.02 (Cu), 0.10 (Fe), 1.05 (Mn), 1.17 (As), 8.5 (Hg), and 0.06 (Se). The model-estimated concentrations in water fall within the range of measured concentrations for Al (99.9%), Ni (99.9%), Pb (97.6%), Zn (100%), Cr (99.7%), Cu (100%), Mn (96.2%), As (100%), and Hg (100%). Due to data availability, evaluations of the redox states are performed for  $Cr^{VI}$ ,  $As^{III}$  and  $As^V$ , and those of the organic-inorganic states are conducted for monomethyl arsenic (MMAs) and methylmercury (MMHg). The  $C_{ratio}$  of the dissolved  $Cr^{VI}$  is 0.23 and 0.16 in water and soil, respectively. The estimated concentration fractions of the dissolved  $As^{III}$ ,  $As^V$ , and MMAs in water are 0.25, 0.67, and 0.07 respectively, as compared to the measured values of 0.45, 0.54, and 0.01, respectively. The estimated concentration fraction of the dissolved MMHg in water is 0.06, which is comparable to the measured value of 0.1. The difference in mean concentration between model and measurements are within the 1 order magnitude, which demonstrate the feasibility of the model developed in this study. Our future study will conduct further evaluations and uncertainty analysis of the model.

### 3.23.P-We200 Effects of Elevated CO<sub>2</sub> and Temperature on Physiological Status of Well-watered and Drought-stressed *Brassica napus* Grown in Cd Contaminated Soil in Relation to Their Phytoextraction Efficiency

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Climate conditions, such as elevated CO<sub>2</sub>, warming, and drought, may have a significant positive or negative impact on plants growing in heavy metal polluted soil. However, the mechanisms involved in Cd accumulation under changing climate conditions have received little attention, and the presence of Cd in growth media at the same time combined with all these climate change-related variables has not yet been investigated. Therefore, this study aimed to assess Cd phytoextraction efficiency in well-watered and drought-stressed *B. napus* plants grown under the ambient CO<sub>2</sub> and temperature conditions (ATC, 21/14°C, 400 ppm CO<sub>2</sub>) and elevated CO<sub>2</sub> and temperature conditions (ETC, 25/18°C, 800 ppm CO<sub>2</sub>). The underlying physiological mechanisms underpinning the obtained results were investigated by studying the Cd (0, 1, 10, 50, and 100 mg kg<sup>-1</sup>) effect on *B. napus* photosynthetic performance and nutritional status. The visible leaf lesions, growth retardation, reduction in gas exchange, ChlF-related parameters, and disturbed balance of mineral nutrients were largely seen only in Cd-50 and Cd-100 treatments but were much more pronounced under ATC conditions at both soil moisture levels. The most important pathways by which Cd affected *B. napus* photosynthetic efficiency were severe Cu deficiency in shoots, resulting in decreased photosynthetic electron transfer between the cytochrome b6f complex (Cytb6f) and photosystem I (PSI) due to a lack of plastocyanin, and the inactivation of some photosystem II (PSII) reaction centers and ferredoxin-NADP<sup>+</sup>-reductase (FNR). There was no significant difference in phytoextraction efficiency between ATC and ETC conditions in drought-stressed plants. Meanwhile, in well-watered plants, ETC conditions resulted in significantly more Cd accumulation in Cd-50 and Cd-100 treatments. However, only the Cd-50 treatment significantly increased soil Cd removal rate (to 65%), which showed the opposite trend as BCF and had a strong positive relationship with shoot DW.

### 3.23.P-We201 Elemental Analysis of Co-Produced Water from the Danish Chalk Reservoirs: Trace Compounds in a Complex Matrix

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When crude oil is produced, large amounts of water are co-produced along with the raw material. This produced water (PW) is highly saline and contaminated by crude oil from the geological formation and production additives. Thus, it is heterogeneous and chemically complex. Since this water is being discharged into the sea at most current offshore production sites, reaching “zero harmful discharge” requires stringent produced water management and improved monitoring and toxicology evaluation. Trace elements are often challenging to measure in formation water brine and thus are hard to quantify reliably in many cases. This work aims to characterize the elements present in low ppm and trace amounts for an improved environmental impact assessment and overcome the analytical challenges of a hypersaline and carbon-rich water sample.

This study presents a workflow for trace element analyses of complex aqueous samples. Some elements have primarily been targeted in suspended particulate matter (SPM) and characterized using EDX-SEM. Then ICP analyses have been focusing on Ba, Sr, Fe, Mn, and more environmentally concerning elements like Cu, Cd, Pb, As, Sb, and Se. Additionally, Hg content determination with CV-AAS has been investigated. The overall concentrations variation within different oil platforms and sampling dates has also been evaluated. To improve plasma stability and mitigate the matrix effect within ICP methods, a matrix-

matching solution for blanks, calibration standards and quality checks has been implemented. A hydride generator introduction system has also been adopted to analyze As and Sb, which cannot be reliably analyzed with standard ICP-OES/MS techniques. The presentation will include the initial results of these analyses of produced water from the post-separator discharge point of several wells from Danish North Sea offshore platforms.

### 3.23.P-We202 Evaluation of Cu-toxicity from Diverse Sources in Agricultural Topsoils of Central Chile

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Topsoils of agricultural areas in central Chile may become polluted by copper (Cu) from several sources as Cu smelter emissions, mine tailings dispersal, and excessive applications of Cu-based pesticides. The long-term exposure of these soils to Cu may decrease their agricultural value. However, few studies have evaluated the increase in Cu levels in topsoils of the area and evaluated their Cu-toxicity effect. Therefore, the aim of this study was to evaluate the effect of field-collected agricultural soils polluted by different Cu sources using a standard laboratory phytotoxicity test. Thirteen agricultural soils were collected from central Chile from sites with suspected Cu pollution. *Lolium perenne* seeds were sowed in each soil (five replicates) and after 4 weeks of cultivation, plants were harvested. Physicochemical characterization of topsoils and chemistry of pore waters were also performed. The shoot length was measured as plant response indicator. Simple and multiple linear regression were carried out to evaluate the effect of soil physical-chemical properties on plant growth. A significative linear regression model ( $p < 0.05$ ;  $R^2 = 0.32$ ) indicated that total soil Cu had a negative effect on shoot length. The best significative multiple regression model obtained ( $p < 0.05$ ;  $R^2 = 0.50$ ), showed the negative effect of total soil Cu on shoot length, but a positive effect of soluble zinc (Zn), organic matter (OM), and nitrogen (N). These results suggest that Zn may alleviate copper toxicity due to competition between  $Zn^{2+}$  with  $Cu^{2+}$  at root absorption sites. The positive effect of OM may be explained by its capability for Cu adsorption, thus being less bioavailable for plants. We conclude that regardless of the Cu source, the increase of Cu from anthropogenic sources has a negative effect on plant growth and that Zn and OM may alleviate this effect. Nevertheless, more studies are needed to confirm the alleviating effect on the negative effect of Cu in plant growth.

### 3.23.P-We203 Exposure to Mercury and Fish and Shellfish, Seafood: The 2016 Korea National Institute Environmental Research Survey

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Mercury is an environmental pollutant widely present in the environment, and humans are generally exposed to mercury in three forms ( $Hg_0$ ,  $Hg_2^{+}$ , and  $Hg^{2+}$ ), and it is known that the body absorbs a lot of mercury through fish intake. We assessed the association between exposure to mercury and fish and shellfish, seafood. Data were derived from the National Institute Environmental Research Survey (2009). This cross-sectional study was investigated survey data from 2009 to the 3rd anniversary. It calculated national statistical data on the level of exposure to environmental harmful substances in the body, and provides basic data for establishing environmental health policies by continuously analyzing the environmental health status of national and regional units, the temporal and spatial distribution, changes, and influencing factors. We compared the level of mercury and food intake, investigated  $p$  for trend mercury according to the intake. The higher the frequency of fish intake, the higher the mercury concentration. Correlation of blood mercury and frequency of fish intake was statistically significant. Compared with the lower group, the higher group by median of blood hg had significantly higher risk by frequency of fish (odds ratio, 1.40; 95% CI, 1.332 to 1.472) and shellfish (odds ratio, 1.188; 95% CI, 1.128 to 1.251) intake. The geometric mean values of mercury in eating shark meat group were 5.264  $\mu g/L$ , which were higher than those in a group that does not eat shark meat (2.793  $\mu g/L$ ). The blood hg had significantly association with frequency of fish and shellfish intake.

### 3.23.P-We204 Fate of Heavy Metals in Shooting Range Soil: Distribution and Phytotoxicity

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Soil contamination with heavy metals still is an environmental concern around the world. Shooting activities play an important role in increasing soil contamination with heavy metals. The outdoor shooting ranges are typically contaminated with various heavy metals, such as Pb, Cu, Zn, Cd, Ni. The scale and amount of contamination depends on shooting activity and soil characteristics. The main contaminant is Pb, as it usually is the main component of a bullet. The aim of the study was to examine the soil contamination with Pb, Cu, Cd, Mn in different types of Lithuanian shooting ranges (practical, small-bore, clay target) and vertical distribution of heavy metals in soil. Study shows that soils were heavily contaminated with Pb; total Pb concentrations exceeded limit concentration in Lithuanian soils (100 mg  $kg^{-1}$ ). Pb was typically concentrated in the back stop berms of practical and clay target shooting ranges and at the end of the shooting area (target zone) of small-bore shooting ranges. Heavy metals and metalloids accumulate in the soil and can migrate along the food chain, adversely affecting the quality of the soil environment and agricultural products, and ultimately harming animal and human health. Understanding the distribution of pollutants in soil and identifying areas at high risk of pollution is therefore the basis for preventing and controlling soil pollution. The aim of phytotoxicity study was to determine the effect of military firing range soil on the growth of sunflower (*Helianthus annuus*) and oat (*Avena sativa*). Lead and copper accumulated most in the roots and shoots of the plants. The highest nickel translocation factor (TF) and bioconcentration factor were found in oats grown on shooting range soil, while zinc - in sunflower. Lead and copper had the lowest TF in plants compared to other metals.



### 3.23.P-We206 Geochemical Modelling of Heavy Metal Removal in Wastewater Using Vermiculite

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The presence and corrosion of zinc from alloys, galvanized steel structures' surfaces, and within soils may induce its leaching into the water collection system and surface waters, which in turn brings the concentration above permitted limits. Collection pond water with zinc concentrations above regulatory limits cannot be discharged into water bodies and into the wastewater collection systems. There is a need for cost-effective options to meet such compliance requirements for greenhouse growers who find elevated zinc concentrations in their waters. Among the options for treating heavy metals contaminated water, selective adsorption using different materials has shown promising evolution and excellent results. While treatment options are available, the effectiveness of treatment is usually investigated by short-term batch experiments. Geochemical modeling is one of the promising tools for verifying and predicting long-term effects of selective adsorption using sorption materials. It may contribute greatly to select best suitable solutions for remediating contaminated sites. In this study, a geochemical model, using vermiculite as a sorbent for zinc removal in aqueous solution, was built. The model was tested against experimental data obtained from previous studies to replicate the environmental conditions and to validate the methodology. Zinc adsorption into vermiculite under more alkaline condition is shown to be efficient and cost-effective, and our modeling results are in line with experimental studies. For the range of pHs expected for greenhouse wastewater, the behaviour of zinc adsorption on vermiculite was very similar, which means it may not be necessary to chemically alter the pH to obtain a good adsorption ratio.

### 3.23.P-We207 Health Risks Associated with Heavy Metals in the Well-water of Palakkad, Kerala, India

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Water is indispensable for the survival of human beings. The presence of heavy metals in water is a matter of serious concern. Ingestion of heavy metals can lead to severe health consequences. The study aims to estimate the heavy metal concentrations in groundwater wells and the associated health risks. The well water samples were collected from various locations across Palakkad district in Kerala, India. Concentrations of Cd, Pb, Cu, Fe and Cr were estimated using Flame Atomic Absorption Spectrometer and compared with the permissible limits as per Indian and WHO Standards. Using Monte Carlo method, the probabilistic non-carcinogenic risk assessment was carried out following the USEPA health risk assessment guidelines. The parameters determined for health risk assessment are the hazard quotient (HQ) and Hazard Index (HI). Except for Copper, all the other analyzed heavy metals were present in concentrations higher than the limit prescribed by IS code for drinking water. The concentrations were found in the order of  $Cd > Pb > Cr > Fe > Cu$ . Fe does not contribute to any possible health risk to the local population. The risk caused by cadmium is very high, with an HQ of the order of  $10^{+02}$ . Sensitivity analysis suggested that exposure duration and concentration are the two influential parameters in calculating the risk. Pb and Cd are probable human carcinogens, but the effect of carcinogenic risk is established only for the inhalation route. Hence the carcinogenic risk was not determined in this study. The present study will help create awareness among the public and the local government on the exposure to heavy metals through drinking water in the study area, where groundwater is the major source of water for meeting domestic demands.

### 3.23.P-We208 Identification of Refinery-originated Metals in River Sediments from Widespread Abandoned Mining Area using Stable Zn and Pb Isotopes

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In order to identify the sources and transport pathway of metals (Zn, Cd and Pb) in river sediments around the Zn refinery, total 58 river sediments samples and potential metal contaminants (ores, Zn concentrates used in refinery, smelting waste and abandoned mine tailings), were analyzed for metal concentrations and stable Pb and Zn isotopes.

Since sediments were already contaminated from abandoned mine tailings through the river, sediments around the Zn refinery would be a admixture by smelter-related substances (imported ores, smelting dust and smelting waste) to tailing contaminated sediments. As the smelter-related materials could be differentiated from the tailing contaminated sediment, the contribution of smelter-related materials could be quantified by Pb isotopes. From the distribution of  $\delta Zn$  in river sediments, two types of smelter-derived pollutants (groundwater and dust contaminated soil), and tailings contaminated sediments could be differentiated and quantified for the contribution of three sources for highly contaminated sediments around the Zn refinery.

### 3.23.P-We209 Investigating the Metal Leaching Potential of Copper-rich Metal Ore Processing Waste Treated with a Deep Eutectic Solvent

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Metal ore processing wastes (tailings) are a mining by-product consisting of residual processed rock after the separation of valuable commodities. It is estimated that upwards of 8 billion tonnes of tailings are produced annually. Inadequate storage of tailings can lead to environmental problems such as acidic mine drainage, metal pollution or tailings dam collapses. Tailings are also a potential source of easily accessible metals. A new class of novel solvents known as Deep Eutectic Solvents (DES) have recently been employed to re-mine tailing deposits. DES are related to ionic liquids and contain both a hydrogen bond acceptor and a hydrogen bond donor, mixed together to form a liquid at room temperature. DES are an attractive alternative for mineral extraction due to their high tunability, cheap components, low (eco)toxicity and lower operating temperatures compared to traditional extraction methods. The environmental fate of DES when applied to tailings has not yet been investigated in any context. To investigate the effect of DES on the liberation of metals to the environment from *in-situ* treated tailings, we conducted

an experiment to compare metal leaching from tailings before and after treatment with DES. Samples were collected from a copper mine tailings storage facility in the Philippines and a sub-sample of tailings were leached using a choline chloride:ethylene glycol DES at a 1:2 molar ratio. The leached tailings were sequentially washed with de-ionised water at varying solid:liquid ratios. A control sample consisting of un-leached tailings underwent an identical washing process. Water samples were analysed for metals and chloride (as a marker for residual DES). Experimental data show an increase in metal leaching from the DES treated tailings. Elevated concentrations of metals were also proportional to DES concentrations. This suggests that *in situ* treatment of tailings with DES (e.g., in re-mining) may increase metal leaching risks in the remaining substrate, at least in the short term.

### 3.23.P-We210 Metallic Profiles of the Most Consumed Rice Varieties in Spain

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Rice is one of the most consumed staple foods around the world and its trade is highly globalized. Increased environmental pollution generates a large amount of waste that, in many cases, is discarded close to culture fields. Some species are able to bioaccumulate toxic substances, such as metals, that could be transferred to the food chain. The main goal of this study was to evaluate the content of metallic (Al, Cd, Pb, and Cr) and metalloid elements (As) in 14 of the most consumed varieties of rice in Spain. Samples were collected from the most popular Spanish supermarkets in triplicate, taking the same brand for the triplicate. The samples were cooked, and human digestion was simulated by using a standard *in vitro* digestion method. Metallic and metalloid element levels were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), previous called microwave digestion.

The statistical analysis did not show significant differences ( $p > 0.05$ ) in the concentrations of Al, Cr, Mn, and Se between all cooked rice in our study. However, significant differences in levels of Cu, Zn, As, Cd, and Pb within the varieties employed were detected. These differences could be due to the ability of each rice variety to bioaccumulate these metals in combination with their concentration in the croplands. In digested rice, there are significant differences in all metals except Pb due to the different digestibility degree in each rice variety. Different correlations were observed in rice produced in Spain with those imported, indicating that the presence of metals and metalloids in rice is mainly impacted by soil composition.

The mean values of the bioavailability of the metallic elements of all varieties studied decrease in the following order:

As>Al>Ni>Mn>Cu>Cd>Zn>Cr>Se>Pb. Inorganic arsenic was the element with the highest bioavailability (79.09%) in contrast to the bioavailability of Pb (11.9%).

Lastly, the results obtained in the present work approach a more realistic metal and metalloid exposure through rice intake, as the cooking and digestion procedures were taken into account, which makes a more realistic risk assessment, performed in most of the cases with measurements obtained in raw material. Thus, performing studies of digestion and bioavailability of these foods in raw, to perform a better risk assessment of these pollutants.

### 3.23.P-We211 Multiple Biomarkers Response of *Astyanax lacustris* (Teleostei: Characidae) Exposed to Manganese and Temperature Increase

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Manganese ( $Mn^{2+}$ ) is an essential metal for living beings, acting in the metabolism of amino acids, lipids, proteins, and carbohydrates. This metal is essential for fish growth, vertebral development, and metabolism. However, anthropogenic activities, such as the release of industrial effluents, have contributed to the increase in the concentration of Mn in the aquatic ecosystem.

This increase can affect the health of organisms since high Mn concentrations can cause neurological dysfunctions and genotoxic, biochemical, and behavioral changes. In addition to the increased concentration of metals in water bodies, there are other environmental stressors, such as sustained temperature increase. The simultaneous presence of thermal and chemical stressors can interact and alter the body's ability to produce adequate defense responses to stress. Thus, the present study aimed to evaluate the toxicity of Mn (6.65 mg/L) at different exposure times (96 h, 7, 14, and 21 days) and evaluate its possible toxic effects on the fish *Astyanax lacustris* (Characidae) through multi-biomarkers and the maximum critical temperature (CT Max). The results show increased Mn accumulation (liver and gills) with increasing exposure time. The glutathione S-transferase (GST) activity showed differences in the group exposed to Mn for 96 h compared to those exposed for 21 days. The acetylcholinesterase (AChE) activity increased in the fish exposed for 7 days compared to the control group. On the other hand, no genotoxic changes were observed. The CT Max showed that the loss of equilibrium of 50% of the fish occurs at a temperature of 39°C, with and without the Mn presence. Furthermore, the catalase gene expression (oxidative stress) did not show alterations. Therefore, the present study results suggest that under natural environmental conditions, *A. lacustris* can accumulate Mn in liver tissue in exposures more significantly than 14 days and gill tissue in short-term exposures (96 h). Although there was an accumulation of Mn in the gills and liver, these accumulated concentrations were not enough to cause intense damage to the specimens of *A. lacustris* since no alterations were observed in the catalase gene expression nor the presence of genotoxic and expressive alterations in the studied enzymes.

### 3.23.P-We212 On the Understanding of Trace Metal Sorption by Phytoplankton in Coastal Environments: Preliminary Results from the AMALGAME Project

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Anthropogenic activities, associated with climate change, influence the physical and chemical properties of aquatic systems, particularly in coastal areas which are the receptacle of continental emissions. Among these emissions, trace metals are persistent and ubiquitous contaminants that are highly reactive, playing critical roles in the functioning of ecosystems. A large part of trace metals is bioavailable and at the base of the marine trophic chain, numerous algal species are able to sequester considerable amounts of these elements from the dissolved phase. Therefore, phytoplankton can exert a control on trace metal concentrations and affect biogeochemical cycling in marine systems. As decaying and settling particles, phytoplankton cells also represent an important vehicle for the vertical transport of trace metals in the water column through cell sinking into deeper waters and/or ingestion by consumers in surface waters. Although phytoplankton communities may play a key role in the geochemical cycling of trace metals in coastal ecosystems, the precise quantification of trace metal concentrations in microalgae remains difficult in environmental studies. Indeed, the collection of Suspended Particulate Matter (SPM) as “bulk material” make difficult the distinction between biogenic and abiogenic origin of trace metals. This technical issue limits the existing knowledge for regulatory elements such as cadmium, copper, or lead, which are now accompanied by emerging trace metals such as Platinum Group Elements (PGEs). One of the main objectives of the AMALGAME Project aims at developing and optimizing methodologies to quantify trace metal levels associated with microalgae, including advanced microscopy techniques such as Scanning Electron Microscopy (SEM) combined with Energy Dispersive X-ray Spectroscopy (EDS). Preliminary results of this study will be presented and discussed.

### 3.23.P-We213 Perspective on Grey Wolves as Sentinel Species of Ammunition-Derived Lead Pollution in Terrestrial Ecosystems

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The exposure route of lead (Pb) ammunition has been extensively studied in avian predators and scavengers, yet only few studies exist on this pathway for mammals. But ingestion of retained ammunition in strayed prey and discarded kills or viscera also poses a periodically high exposure risk to scavenging and predatory mammals. These periodic events can be easily overlooked when monitoring blood or soft tissue due to the relatively short biological half-life of Pb (several weeks in blood to few months in liver tissue). Since Pb follows the same metabolic mechanisms as calcium (Ca), it is mainly stored in mineralized tissues, where it is retained for up to 30 years. Young and growing vertebrates have higher absorption rates of Pb as adults due to their high Ca demand and are more affected by its detrimental effects. Lead trapped in bone, reflecting the sum of life-long exposure events, is considered inert but can be remobilized during periods of physiological stress (e.g., pregnancy, lactation, Ca deficiency).

With the return of grey wolves (*Canis lupus*) to Germany since 2000 and the detailed monitoring on causes of death and diseases established by the IZW, there is an excellent opportunity to systematically study the contaminant burden. Grey wolves are highly opportunistic apex predators and thus prone to bioaccumulation of contaminants, but large carnivores are seldom analysed for Pb accumulation in Europe.

As part of a pilot study, Pb levels in small cortical bone samples (femurs) from 32 grey wolves found dead between 2017 and 2021 were analysed using atomic absorption spectrometry. Lead levels ranged from 0.29 ppm d.w. to 9.51 ppm d.w. ( $\bar{x}$ : 1.38 ppm;  $\tilde{x}$ : 0.99 ppm). The highest concentration was found in an adult male that died in a traffic accident. Necropsy reports showed no metal particles, so dietary exposure can be assumed.

The results indicate that grey wolves in Germany may be exposed to elevated Pb levels, however, further investigation is needed to establish baseline levels and to elucidate the sources of Pb. We propose that elevated Pb levels are more likely to occur in regions with higher hunting activity, thus providing indirect evidence of the use of lead-based ammunition. Depending on the property situation and restrictions of lead-based ammunition we would expect lower lead levels in state-owned hunting areas. It is of high importance to examine long-term exposure trends to evaluate if regulations on lead-based ammunition are effective.

### 3.23.P-We214 Polyethylene Microplastics Interactions on Microbial-mediated Hg Removal – Implication for Naturally Occurring Hg Detoxification

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The pollution of coastal areas has become an increasingly recurrent problem, where contaminants such as mercury (Hg) and microplastics (MPs) threaten aquatic organisms and even human health. The presence of Hg in the marine environment is a worrisome problem due to methylmercury formation with its subsequent bioaccumulation and biomagnification. Microplastics may act as a vector for other pollutants such as Hg, which tends to get adsorbed to its surface, increasing the overall toxicity, and also as an attachment surface for microorganisms. Microorganisms are the key player in Hg-biotransformations.

The main goal of this study was to investigate the interference of polyethylene microplastics (PE-MPs) on Hg detoxification processes mediated by microbial communities of a Hg-contaminated marine ecosystem. Microbial communities and *Pseudomonas* strains, isolated from a highly Hg-contaminated area of Tagus Estuary, were exposed for 6 days to PE-MP, Hg (1-2 ppm), and a mixture of both pollutants. A control containing Hg, PE-MPs, and a mixture of both was run. After 1, 3, and 6 days, supernatant and MPs were collected to: (1) quantify the extracellular polymeric substances (EPS); (2) to determine the total Hg concentration (HgT) through atomic absorption spectrometry; (3) to analyze structural changes in the properties of the polymer by FTIR analysis, and (4) to assess the acute toxicity of the supernatant (Microtox acute test). All the collected samples showed the presence of EPS associated with the PE-MPs, which indicates that there was biofilm formation. The HgT was lower in treatments

with the microorganisms and Hg, indicating Hg-removal mediated by these microorganisms. The treatments containing PE-MPs and Hg exhibited higher HgT, showing interference of PE-MPs on Hg-removal by these microorganisms. The same was obtained for the acute toxicity results, showing higher toxicity associated to the presence of PE-MPs in the incubation medium. These interfere with the Hg-removal and supernatant toxicity may be related to the formation of biofilm on the surface of PE-MPs and as well the adsorption of Hg to the surface of these microplastics, which decreases its availability to be removed. The PE-MPs did not suffer any degradation. Through this study, it is possible to conclude that PE-MPs interfere with the removal process of Hg mediated by Hg-resistant microbial communities, affecting the overall toxicity associated to the media.

### **3.23.P-We215 Predicted Environmental Concentrations of Metals and their Environmental Risks, Anticipated by Concurrent In-Water Hull Cleanings of Ship's Biofouling in an International Harbor**

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When organisms grow on ship's hull, the drag they produce slows the ship's movement, increasing the consumption of fuel and leading to higher emissions of greenhouse gases. Furthermore, the growth of these organisms can lead to a transfer of invasive species from one place to another, which could lead to the destruction of local ecosystems. By periodic in-water cleaning (IWC) of ship's hull, the biosecurity risk can be mitigated, and the ship's fuel efficiency can be improved. However, during the cleaning of the hull, the active substances (e.g., heavy metals) in the antifouling paints can be released and introduced directly into the marine ecosystem. The active substances can be a source of hazards to marine organisms in the environment. To understand the risk posed by IWC, environmental concentrations of metals released during IWC were predicted using a steady-state 2D integrated hydrodynamic and chemical fate model in one of the world's largest international harbors and compared to no-effect concentrations to characterize risk of target chemicals. Release rate of metals from hull surface was calculated based on the information collected during actual IWC cases, which include metal concentrations in the effluents, effluent volumes, and hull areas. A release rate under realistic worst-case scenario was used for model prediction as a conservative approach. The predicted environmental concentrations were proportional to the number of cleanings and the size of ships under cleaning. A single hull cleaning of any size seems not to clearly damage the marine environment. However, when considering concurrent IWC of ships berthing in the harbor, the predicted environmental concentration exceeded local standards set for the protection of marine environment. Predicting environmental concentration only for single cleaning cannot appropriately assess the environmental risk in a given site, where multiple operations are expected. The daily demand for in-water cleaning at the site should be estimated and this demand needs to be accounted for risk assessment. In-water cleaning should only be allowed if the predicted environmental concentrations is within the threshold of the environmental capacity. The risk assessment framework proposed in this study can be used for environmental management purposes in port areas expecting frequent and multiple in-water cleaning activities.

### **3.23.P-We216 Regional Exposure Assessment of Metals in the Aquatic Freshwater Environment**

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Environmental release rates of metals are expected to vary over time due to new uses and changes in used/manufacturer volumes, but also due to improvement of emission-reducing technologies and external conditions like climate change. As a consequence, previously collected regional ambient concentration levels that were used in a regulatory context (e.g., for exposure scenario development under REACH) may not be relevant anymore for current or future situations. An update of these regional metal concentration levels in the aquatic environment is part of Eurometaux's currently ongoing comprehensive "Metals environmental exposure gathering program" (MEED-program), covering today's and expected needs for tomorrow to comply with the Zero Pollution Ambition and biodiversity objectives.

Recent monitoring data (period: 2017–2021) of dissolved metal concentrations in EU surface freshwater systems were extracted from EEA's database on the status and quality of Europe's rivers, lakes, and groundwater bodies (Waterbase). Data sets for 18 metals, covering up to 20 countries, were compiled and represented the basis for the derivation of country-specific reasonable worst-case (RWC) ambient PECs. The followed calculation methodology is stooled on the data treatment procedures that have been laid out in Guidance Documents for environmental risk assessment in the EU and includes an outlier analysis to minimize the impact of local point sources when predicting a representative ambient regional concentration.

Only quality-screened measured samples were considered. Data were categorized into sub-datasets (river basin, a specific waterbody, or an individual sampling location) that were sufficiently large to derive a meaningful 90<sup>th</sup> percentile; the median of all 90P-values represents the ambient RWC-PEC.

Overall, the determined RWC-ambient PECs were within the same order of magnitude as those that were derived in the past (2000-2010)-period. Observed differences in country-specific RWC-ambient PECs for individual metals are likely caused by factors such as specific geological conditions, (over-)representation of sampling locations that represent major rivers affected by industrial activities (e.g., Rhine, Danube). For most metals, the calculated RWC-ambient PEC is typically situated between the 50<sup>th</sup> and 90<sup>th</sup> percentile of known baseline levels (FOREGS-dataset), making the latter a potential reference for estimating the RWC-ambient PEC for metals lacking monitoring data.

### **3.23.P-We217 Removal of Carcinogenic Metals in Tobacco Obtained From Waste on Roadside**

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## India

Smoking habits is a major risk factor for a number of diseases including cancer, chronic obstructive pulmonary and cardiovascular disease. IARC states that human carcinogens include lead, cadmium, arsenic, nickel, and chromium. Cigarettes include these elements, however the amount varies depending on the region where tobacco is grown, how fertilizer is applied, the type of plant, etc. This study attempts to evaluate the levels of these metals in cigarettes sold in India. The tobacco filling was taken out of from different source then independently weighed and homogenized. Samples were desiccated and then put through aqua regia. Atomic absorption spectrometry analysis was used to confirm the presence of heavy metals. Metal oxide doped Prussian blue analogues were synthesized using *A.indica* leaves extract was used for the removal of these heavy metals. The physicochemical properties of all nanoparticles were investigated by different spectroscopic and microscopic analysis. Development of alternative photocatalyst is on –demand to avoid bioaccumulation of particular metallic structures. Co-ordinated complexes like hexacyanoferrates and cobaltates of Zn are completely safe substances for humans, comparatively stable and non-toxic. This leads to creation of defects, an increase in the trapping rate, and enhanced the photocatalytic activity of photocatalyst. Thus, such a photocatalyst could be used as a promising material for application in industrial wastewater treatment processes.

### 3.23.P-We218 Selenium Accumulation in Sediment in Las Tablas de Daimiel National Park: Identification of Areas of Greatest Risk for Biota

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Tablas de Daimiel National Park (TDNP) is a Mediterranean wetland in the central Spanish plateau. Despite its declaration as a Biosphere Reserve and Ramsar site, its persistence is threatened by desiccation due to groundwater overexploitation and by pollution from untreated wastewater treatment plant effluents. Previous studies of our group have shown high selenium levels in waterbirds and fish from TDNP that can be of concern. In addition, several studies have shown that the soil or sediment of TDNP and parts of the Cigüela basin upstream are seleniferous. This scenario may be similar to that observed in a Kesterson Swamp wetland located in the San Joaquin Valley in California, where run-off of irrigation water used in seleniferous soils caused Se intoxication of waterbirds nesting in that wetland. The objective of this study is to map the Se levels in soils and sediments from TDNP to determine the influence of geological and hydrological variables on Se pollution in the Park. We collected 188 soil or sediment samples in a systematic manner to cover the entire wetland surface. Se levels were analysed with inductively coupled plasma with mass spectrometry (ICP-MS) in samples previously digested with nitric acid and peroxide hydrogen. Periods of drought and flooding can have a significant effect on Se cycles given their influence on the oxidation-reduction reactions that are involved in the formation of different Se species. With this mapping, we will define the areas of greatest risk for birds and other organisms and the potential sources of Se. Further research will be conducted to study the speciation of Se in sediment and water of TDNP and its relationship with the hydrologic cycles of the wetland.

### 3.23.P-We219 Spatial and Temporal Analysis of the Risks Posed by Metal Contamination in Coastal and Marine Sediments of Bahrain

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Nine metals including Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were analysed from sediment samples collected from 29 stations since 2007 from Bahraini waters. Within this study, it was investigated whether concentrations of these determinants are at concentrations above internationally established Assessment Criteria (AC). The majority of sites were considered not to pose a toxicological risk in terms of metal contamination. Where breaches occurred, they were mainly from historic samples related to Cr, Cu, and Ni contamination. A trend assessment revealed that out of 59 significant trends, 36 were downwards and 23 upwards, indicating that some determinants like Al, Zn, and Ni are improving strongly across some sites, whilst areas associated with industrial activity still see some increasing trends for Al, Cd, Pb, and Zn.

### 3.23.P-We220 State-of-Science Review of the Assessment of Chemical Contamination of Abandoned Mines in Nigeria

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Abandoned mines pose serious risks to the environment, through the release of pollutants to land, rivers, and air. Thus, harming plants, aquatic organisms, other animals and may impact human health in areas of high pollution. In Nigeria, over 1200 abandoned mine lands (AMLs) have been identified. To date, only a few have been reclaimed due to the high costs. Many non-reclaimed AMLs have pond waters that are being used for irrigation, fishing, domestic and industrial purposes exposing humans to risk of significant harm.

A systematic evidence map was carried out to review chemical assessment methods of legacy mines environment. Preliminary results revealed that focus has been on heavy metal analysis using routine methods, with little insights into the behaviour and fate of these contaminants in this environment. This is largely due to lack of access to the current state of the art technology in identifying contaminants that are comparable to what is obtainable in other developed nations of the world.

To address these knowledge gaps, an integrated impact assessment methodology for chemical contamination emanating from abandoned mines in Nigeria study will be developed to identify and quantify the chemical contaminants and the interactions between the different media around the mines to ascertain the extent, fate, mobility and impacts of the contaminants on receptors accounting for the risk of exposure to multiple pollutants via multiple sources.

**3.23.P-We221 Trace Metals Accumulation with Age in Bats from a Moderately Polluted Area in North-Eastern Ukraine**  
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Being particularly long-lived small mammals, bats are especially endangered with metal poisoning due to the tendency of some metals to accumulate in internal tissues throughout the lifetime. The impact of trace metals on bat physiology is generally not well-studied, and effects on bat populations are virtually unknown. In our current research we studied how trace metal concentrations change with an age in different tissues of bats.

Bat carcasses have been obtained from the bat carcasses storing collection at the Bat Rehabilitation Center (BRC) of Feldman Ecopark in Kharkiv, Ukraine. The extracted internal samples were entire organs (liver, kidney). Our focal species is *Pipistrellus kuhlii*, which lives for years in one place and is strongly associated with urban environments and can benefit from urbanization. All samples were analyzed for concentrations of Pb, Cd, Cu, Zn using atomic absorption spectrophotometer AAnalyst 200 and PinAAcle 900Z (Perkin Elmer). Osteochronology was applied to identify the exact age of individuals, using cross sections of the upper canine tooth of each individual (Leica CM1950 cryostat). For age identification number of dentine rings in the sections were counted.

The age of all 40 individuals of *P. kuhlii* was identified. Majority of individuals were this-year-born and one-year-old. The oldest bats were six years old. To test whether metal concentrations in the kidneys and livers of bats depend on age, regression analysis was used. For each metal the regression lines were compared between the two tissues. Statistically significant ( $\leq 0.05$ ) increase in metal concentration with age was found for Cd ( $R^2 = 16.05\%$ ), Pb ( $R^2 = 14.38\%$ ), and Zn ( $R^2 = 10.45\%$ ); for Cu the relationship was marginally significant ( $R^2 = 7.40\%$ ,  $p=0.06$ ). Cadmium accumulated with age only in the liver ( $p = 0.007$ ), but not in the kidney ( $p = 0.8$ ).

Assuming that the age structure in our sample represents the age structure in other colonies of *P. kuhlii*, in which indeed the majority of individuals are young and only several are old, the results would have broader applicability and may indicate that in more polluted environments the accumulation of metals in internal organs can lead to chronic effects in older bats. On the other hand, metal concentrations found in this study, even in the oldest individuals, did not reach concentrations at which toxic effects are expected.

**3.23.P-We222 Two Thousand Years of Heavy Metal Pollution in Southwest Europe: A Study Through The Cattle Ranch of Cartagena (Southeastern Spain)**

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Environmental contamination by heavy metals in mining areas affects human health. The intake of products from livestock that graze in mining areas is one of the pathways for contaminants into human diet. There are several studies on the greater exposure to metals and metalloids in small ruminant herds and wildlife, especially in areas of extensive mining exploitation. However, studies carried out in a diachronic perspective do not predominate, on broad chronological arcs in which anthropic contamination by heavy metals has varied depending on the greater or lesser exploitation of the territorial metallic wealth according to diverse cultural parameters. Evidence of diverse rates of contamination by these metals have recently been corroborated in above mentioned chronological arc on the soils immediately adjacent to ancient Carthago Nova (present-day Cartagena, southeast Spain).

This study show bone concentrations of Pb, Cu, Zn, Cd, As, Sr, Fe, Cr, Ni of 44 goats (*Capra hircus*) recovered in the excavations of the Roman Theater of Cartagena and in the nearby Iberian settlement "Los Nietos", located on Mar Menor's coasts (Southeastern Spain). Both records cover a wide temporality, from 5th century B.C. to 18th century A.C., to which is added its location in a region rich in silver minerals, concentrated in the Cartagena-La Unión mountain range and the Mazarrón mining area. Both areas show archaeological mining-metallurgical evidence of silver exploitation, at least from the 7th century B.C. in relation to the presence of Phoenicians and Punics in coastal settlements, with an important boom in Roman and Byzantine times, maintaining activity until the 20th century. Analyses were done using an Inductively Coupled Plasma Mass Spectrometry (IPC-MS).

The results of Pb show traces of contamination in Iberian period (214.6 mg/kg) and, to a lesser extent, in 3<sup>rd</sup> century, Roman period (18.03 mg/kg), with a decline until Byzantine period (6th-7th centuries, 4.66 mg/kg), and a notable increase in Islamic period (12th-13th centuries, 117.87 mg/kg), reaching again very low levels in modern times (18th century, 10.52 mg/kg).

Comparison of these results with others from human remains from same periods and sedimentary cores of Cartagena's subsoil allow us to present an almost linear image of the impact that metallurgical activity has had on the inhabitants of Cartagena for more than 2000 years, completing and correcting information from literary and archaeological sources.

**3.23.P-We223 Zinc Sources to Water: An Apportionment Exercise for the EU27**

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Reporting of point and diffuse source emissions of chemicals has been identified as an issue across the globe. Although large emitters report for chemicals through the European Pollution Release and Transfer Register (ePRTR) little is known on a Member State scale regarding diffuse total emissions to water including diffuse as well as point sources. This project focused on estimating

loads to water for zinc for all significant primarily anthropogenic sources, including industry, sewage treatment plants, surface runoff (from traffic, architecture, and atmospheric deposition), septic tanks, agriculture (biocides, biosolids, fertilisers, manures, and atmospheric deposition), mariculture, sacrificial anodes, marine transport (antifoulant leaching) as well as natural processes and sources (atmospheric deposition and soil loss). To achieve this, a combination of European datasets (Eurostat databases), literature and industry data were utilised to generate export coefficients which could be multiplied by activity rates to generate loads.

A total of around 22,500 tons of zinc per annum (tpa) was estimated to enter freshwaters of the EU27 from diffuse sources, mostly associated with natural background concentrations in the soil eroding into the river (~7,000 tpa). Approximately 3,000 tpa of zinc entered freshwaters from rural area run off, and about the same amount entered transitional and coastal waters combined from sacrificial anodes (~3,000 tpa). Sewage treatment plant effluent, urban runoff directly to water, and other wastewater (intermittent discharges from combined sewer overflows and septic tanks) were in the 1000 to 1700 tons per year range with industrial discharges, agricultural runoff from fertilisers and intensive aquaculture all contributing less than a 1000 tpa. Abandoned mine zinc emissions were estimated to be in the 1000's of tpa, but a lack of data prevented quantification to any degree of confidence. There were differences in predominant sources between countries depending on a number of factors including patterns of use within architecture, size of shipping fleets, and land use.

Where possible estimates of uncertainty were performed, highlighting a lack of confidence in data from abandoned mines and many variables and assumptions relating to urban runoff load estimates. This exercise has provided for the first time a methodology and load apportionment, identifying data gaps and uncertainties which may be refined over time.

### 3.23.P-We224 Copper Toxicity Threshold for *Eisenia fetida* in Copper Pesticide Affected Soil

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Copper-based pesticides (CBPs) have been long used as a fungicide and bactericide worldwide, thus resulting in Cu accumulation in soils. Increase in total Cu concentration in agricultural topsoils may harm soil biodiversity and reduce soil quality. Earthworms have been frequently used in ecotoxicological studies to test the effect of Cu-spiked soils with salts (e.g., CuSO<sub>4</sub>) and also used to test toxicity of Cu mine affected soils collected in the field. However, there are no studies at present using earthworms-based bioassays to evaluate Cu toxicity of agricultural soils contaminated with CBPs. The objective of the study was to determine levels of Cu-toxicity in CBP-polluted agricultural topsoil for earthworms. Fifty orchards' topsoils (0-5 cm) were collected from central Chile (total Cu: 23-566 mg kg<sup>-1</sup>). None of these soils were under the influence of any other source of Cu (e.g. mining) except CBPs. The *Eisenia fetida* avoidance test was used with proposed modifications of Delgadillo et al. (2017). Each field-collected soil was tested with an artificial control soil, which was amended with peat and a 10 g L<sup>-1</sup> solution of NaCl to match the organic matter content and electrical conductivity of each field-collected soil. Estimated effective concentrations EC10, EC25, and EC50 were 57, 117, and 243 mg Cu kg<sup>-1</sup> dry soil, respectively. Results were in accordance with the Cu thresholds for earthworms proposed by Santa-Cruz et al. (2021) of 123 and 282 mg kg<sup>-1</sup> for EC10 and EC50, respectively. We conclude that *Eisenia fetida* is a good indicator of Cu ecotoxicity in copper pesticide affected agricultural topsoil.

### 3.23.P-We225 Characterization of Abrasion-Induced Metal Emissions from Rail Transport into the Environment

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In terms of environmental protection, rail transport is advantageous compared to other modes of transport. Nevertheless, particulate emissions, mainly caused by abrasion, are released into the environment during railway operation. However, only a few studies have been published on the particle size distribution, particle mass and chemical composition of these abrasions. Metal particles, such as copper and iron, are expected to be emitted into the environment as those metals are commonly used in rail transport components (e.g., wheel, rail, brakes, and contact wire). In Switzerland, iron was identified as the main component of abrasion related emissions in rail transport. Similar results were obtained in previous studies, where an increased iron and copper concentration was found in soil samples along the railway tracks. The concentration of these components decreased with increasing distance to the tracks.

The scope of the present work is to obtain detailed information on the particle mass, particle size distribution and chemical composition of the source-specific abrasion emissions. Therefore, extensive test bench investigations of particulate emissions (from wheel-rail, contact wire-pantograph, brakes) as well as field measurements are carried out within the BMDV Network of Experts.

These studies consider particles in the range of a few nanometers up to the size fraction PM10. Based on first results, the determined PM10 emission factors for the wheel-rail contact of an entire train ranges from 0.33 to 5.57 g/km. 83 to 90 % of the PM10 emissions were particles of the PM2.5 fraction and 60 to 73 % were classified as part of the PM1 fraction. Significant higher particulate emissions were found in tight curves than on straight tracks and iron was identified as the main component. The obtained data will be used for dispersion calculations and an evaluation of consequences for the environment.

Additionally, the morphology and the fate of abrasion-related emissions in the environment will be investigated more detailed using SEM-EDX.

Overall results will be used for an initial risk assessment including (eco)toxicological aspects to close current knowledge gaps and to enable a comparison of transport systems.

### **3.23.P-We226 Advances in Methylmercury Effect on Fish: Subcellular Partitioning Associated to Lipid Peroxidation on Northern Pike (*Esox Lucius*) of the St. Maurice River Near Run-Of-River Dams**

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Methylmercury (MeHg) readily bioaccumulates and biomagnifies in aquatic food webs leading to elevated concentrations in fish, which also represents the main pathway of human exposure to mercury (Hg). The construction of hydroelectric power plants with large reservoirs are known to flood terrestrial soil leading to an anoxic environment favoring microbial Hg methylation and food chain transfer for decades after impoundment. Run-of-river (RoR) dams are smaller facilities with a lower water residence time and a smaller surface of flooded areas (called poundage) that may represent a sustainable alternative. Whereas human Hg health issues are well-known, the literature about Hg toxicity to wild fish is less documented. We performed a field study on northern pike with high liver MeHg concentrations (1–51 nmol g<sup>-1</sup> dry wt) from the St. Maurice River (Québec, Canada) affected by two RoR dams, artificial wetlands, forest fires and logging activity. A subcellular partitioning protocol was used to separate the liver into metal-sensitive (mitochondria, microsome/lysosome and HDP – heat-denaturated proteins) and metal-detoxified fractions (metal-rich granules and HSP – heat stable proteins). Results showed that 78% of MeHg was associated to the metal-sensitive fractions suggesting potential deleterious effects. Among the metal-sensitive fractions, MeHg was predominantly found in the HDP fraction which contains important antioxidant enzymes. To link MeHg subcellular partitioning to physiological effect, we measured malondialdehyde (MDA) in livers as an indicator of lipid peroxidation. We observed a relationship between MDA concentration and MeHg concentration in the metal-sensitive fractions with the strongest being associated to the mitochondria ( $R^2 = 0.71$ ,  $p < 0.001$ ). A Se:Hg molar ratio above 1 may protect against Hg toxicity due to their high affinity for each other. In each metal-sensitive fraction, Se:Hg molar ratio were negatively correlated to MDA concentration suggesting that fish with higher Se:Hg subcellular molar ratio yield lower lipid peroxidation levels. This study highlights the importance of combining the subcellular partitioning approach to biomarkers to adequately assess the potential toxicity of MeHg. Lipid peroxidation is not the only effect to occur following metal exposition and other biomarker measurements and omics approaches should be considered.

### **3.23.P-We227 Assessment of the Metal Contents of Dairy Processing Sludge and Municipal Sludge, Including Their Derived Biochar, as Circular and Sustainable Soil fertilizer Components**

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Industrial dairy/milk processing requires effluent management in wastewater treatment systems, from which a considerable amount of waste activated sludge is produced. Similarly, municipal (or sewage) sludge is produced during the treatment of municipal wastewater. The transformation of sludge into biochar is achieved by thermochemical treatment (e.g., pyrolysis) of the dairy/municipal sludge at high temperatures and in an environment deprived of oxygen.

Dairy and municipal processing sludges both contain important organic nutrients such as nitrogen, phosphorus, calcium, and potassium and can therefore be used as a soil fertiliser (biosolids), since it is the most cost-effective way of their disposal. Additionally, biochar produced from dairy and municipal sludge processing, when used as a soil amendment, can increase the total organic carbon, therefore improving the fertility of the soil. Furthermore, the use of high temperatures to produce biochar allows for the removal of harmful bioplastics, organic contaminants and pathogens present in sludge. Nevertheless, both sludge and biochar may contain also significant concentrations of heavy metals, which might be toxic for the soil and surrounding environment.

In this research, a comparison of the compositional analyses of dairy processing sludge and its biochar versus municipal sludge and its biochar was undertaken for the assessment of heavy metal content. The results clearly showed that dairy sludge and its biochar contained very low levels of heavy metals (arsenic, cadmium, cobalt, chromium, manganese, mercury, nickel, lead, zinc), making these waste products good candidates for use as fertiliser components under the Fertilizing Product Regulation (EC2019/1009). On the other hand, municipal sludge and its biochar showed high levels of heavy metals, proving to be potentially harmful for the soil. In addition, calculations on energy requirements for pyrolysis were carried out, showing that the content of solid matter in dairy/municipal sludge waste is critical for making the process of biochar production self-sustaining. A solids content of minimum of 30% in dairy/municipal sludge is required.

In conclusion, the use of dairy processing sludge and its biochar is suggested as a potential constituent of fertilisers over the use of municipal waste sludge and its biochar, which might cause heavy metal contaminations into the soil.

### **3.23.V Sources, Fate, and Effects of Metals in the Environment**

#### **3.23.V-01 Determination of the Influence of Wastewater Effluents on the Heavy Metal Distribution in Sediments in the Kuils River system, Cape Town, South Africa**

**Vernon S Somerset<sup>1</sup>** and Nangamso Mabuya<sup>2</sup>, (1)Cape Peninsula University of Technology, South Africa, (2)Chemistry, Cape Peninsula University of Technology, South Africa

Chemical stress in aquatic ecosystems is increasing and receiving the necessary attention. Urban areas are struggling with wastewater management due to rapid industrialisation and urbanisation, especially in developing countries. As wastewater



treatment facilities fail to meet treatment guidelines, effluent discharged from these facilities pose a threat to aquatic ecosystems and human health. Wastewater from sewage treatment plants contain heavy metals, non-metals and organic compounds originating from industrial and domestic sources. The metals contained in effluents are mainly in the form of suspended particulates and they ultimately accumulate in sediments at the bottom of the rivers and water bodies. At high pH, it will be observed that most metals precipitate in the form of oxides and hydroxides.

The goals of this study are (i) to determine the effect of treated effluent discharged to the Kuils River, (ii) the concentration of Zn, Cu, Pb, Cr, Cd, and Hg in sediments and invertebrates. The list of metals is the most common in domestic and industrial effluent and can have serious impacts on the food chain, ecosystem quality and human health.

### **3.23.V-02 Heavy Metals Associated with Atmospheric Fine Particulate Matter in Cape Town, South Africa**

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Cape Town, South Africa is located at the southern tip of Africa and is surrounded by the Atlantic and Indian Oceans. Cape Town has a Mediterranean climate that is characterised by warm summers and wet winters. In the winter months (June to August) the mean minimum temperature can be as low as 7.1°C (July), while in the summer months (December to February) the mean maximum can rise to 27°C (February). Most of the rainfall occurs during the winter months of June through August. Average precipitation for the region is 515 mm per annum. Cape Town started experiencing its worst drought in 100 years from 2015, with the day zero scenario campaign launched to prevent water shortages, which has seen rainfall dwindle in recent times, due to the impact of global change. The city is also known for its carbon emissions and the threat it poses to human health.

Particulate matter (PM) is known to contain heavy metals in its fine particles, such as PM<sub>2.5</sub> that specifically poses a threat to human health. The aim of this study is to analyse the metal content at a specific air monitoring station to determine the distribution of selected metal concentrations. The soluble metal fraction bound to PM<sub>2.5</sub> and collected on filter membranes are analysed by ICP-MS spectroscopy to find the most abundant soluble metals in the particulate matter. The contribution of soot particles from localised sources, vehicle fuel emissions, are some of the local sources currently investigated.

### **3.23.V-03 The Leaching Behaviour of Rare Earth Elements in Soil Samples Derived from Electronic Waste**

**Martin Makombe** and **Vernon S Somerset**, Cape Peninsula University of Technology, South Africa

The dumping of waste electrical and electronic equipment (WEEE) and its recycling commonly known as “urban mining” has caused undesirable impacts on the environment. Many of these products are rich with rare earth and hazardous elements that end up in rubbish dumps and recycling centres. Harmful metals are exposed and leached into the environment. The fate of rare earth elements (REEs) through the disposal of electronic waste and urban mining into the environment has caused pollution and pose a threat to human health and the aquatic ecosystem. In this work, we report the results of several microcosm studies using conditioned soil to investigate the leaching of REEs in lab-scale experiments. The effective leaching of specific rare earth elements (La, Nd and Dy) were conducted under different experimental conditions. The effects of major variables on REE leaching were evaluated, which included lixiviant type and concentration, time, stirring speed, pH and solid to liquid ratio. The temperature was kept constant during all experiments at 23 ± 2°C. The leaching efficiency of La, Nd, and Dy was found to be significantly dependent on acid concentration and leaching time. The best leaching efficiency was obtained with 1 M HCl with a leaching time of 30 minutes, 300 rpm stirring, 50 g/L solid to liquid ratio. It was found that the leaching efficiency of 69%, 75%, and 77% was achieved for La, Dy, and Nd, respectively. In speciation studies, the results showed that 90% of REEs were obtained from acid-soluble fraction and residual fraction. The study is currently investigating the availability of the fractions, chemical behaviour in soil under environmental conditions, and the uptake of the REEs by biota.

### **3.23.V-04 Heavy Metals and PAHs in Soil from Tyre Pyrolysis Plant located in Egbeda Local Government Area, Nigeria**

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As designed by nature, our environment takes in all we give into it and gives them back to us in another form which could be beneficial or harmful. This study is aimed to assess heavy metals and PAHs in soil from tyre pyrolysis plant at Egbeda, Ibadan, Nigeria. Soil samples were collected from seven strategic locations around the plant. Five selected heavy metals name Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Zinc (Zn) were determined using Atomic Absorption Spectrophotometer (AAS). PAHs were extracted from soil samples through an accelerated solvent extraction system (ASE 200, Dionex, Sunnyvale, CA, USA) with a 1:1 (v/v) acetone/dichloromethane solvent mixture. The results of heavy metals showed that the average concentration of the five metals are As-0.2827 mg/kg, Cd- 0.2177 mg/kg, Cr-0.1726 mg/kg, Pb-0.6753 mg/kg and Zn-1.0232 mg/kg and in decreasing order of abundance ranked Zn > Pb > As > Cd > Cr. One way ANOVA reveals that all the results were significantly different from one another ( $p < 0.05$ ), similarly there was no correlation between all the five metals examined. The degree of contamination and the pollution index of the study site are 13.8 and 2.2 respectively and they implied that the site is moderately contaminated. The PAHs result showed that a total of 18 PAHs were determined with average concentration ranking of Naphthalene –381.25 mg/Kg, 1-Methyl Naphthalene –335.50 mg/Kg, 2-Methyl Naphthalene –236.25 mg/Kg, Anthracene –199.75 mg/Kg, Fluoranthene –173.65 mg/Kg, Phenanthrene –140.00 mg/Kg, Pyrene –78.78 mg/Kg, Chrysene –72.20 mg/Kg, Benzo(a)anthracene –48.78 mg/Kg, Benzo(a)pyrene –45.72 mg/Kg, Acenaphthene –36.68 mg/Kg, Indeno(1,2,3-cd)pyrene –31.26 mg/Kg, Benzo(b)fluoranthene –30.35 mg/Kg, Benzo(k)fluoranthene –27.54 mg/Kg, Fluorene –21.93 mg/Kg, Benzo(g,h,i)perylene –18.51 mg/Kg, Dibenz(a,h)anthracene –15.22 mg/Kg, and Acenaphthylene –12.98 mg/Kg. Seven major carcinogenic and tetratogenic PAHs were detected in slightly elevated levels. One way ANOVA reveals that the concentration of

all the PAHs identified were significantly different from one another, although correlation study indicated that there is strong positive correlation between all the PAHs.

### 3.23.V-05 Insight into Relationships Between Alkali and Alkaline Earth Metals in a Clay Pit Core Sample, with and without Al-Normalization

**Josip Jurković**, *Applied Chemistry, University of Sarajevo, Bosnia and Herzegovina*

Clays are fine particle size (less than 2  $\mu\text{m}$  in size) materials comprised mainly of clay minerals, which are basically hydrated aluminum silicates with associated alkali and alkaline earth elements. The small particle size and complex porous structure result in high specific surface area, which allows strong physical and chemical interactions with dissolved species. Metals can also be incorporated inside of clays structure, which can be made from octahedral and/or tetrahedral sheets. Depending on the composition of the tetrahedral and octahedral sheets, the layer will have no charge or will have a net negative charge. If the layers are charged this charge is balanced by inter-layer cations. The recent researches of metals in clays mainly corresponds to heavy metals, but investigations of light metals, is rather rare. For the investigation of behavior of alkali and alkaline earth metals in a clay samples, 110 sub-samples of cored sample were taken. Total content of potassium, magnesium, calcium, sodium, strontium and barium was determined by means of ICP-OES instrument. Descriptive statistics was conducted by means of maximum, minimum, average and median value. For determining of different behavior of metals correlation and PCA analysis with and without Al-normalization were calculated. The concentrations of metals lie in the next sequence:  $\text{K} > \text{Mg} > \text{Ca} > \text{Na} > \text{Sr} > \text{Ba}$ . The highest correlation between metals was found between Mg and K ( $r=0.842$ ) and K and Na ( $r=0.645$ ). Multivariate statistical analysis distinguished two subgroups: 1) K, Mg, Na, Ba. 2) Ca. After application of Al normalization Ca and Sr showed highest difference in correlation to magnesium. Suggesting a possible different source and behavior. Magnesium is probably part of a clay material, and calcium and strontium are mainly adsorbed onto clay particle.

### 3.23.V-06 Bioaccumulation of Mercury in Barn Owls and their Foodchain

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The fate of mercury as a global pollutant in all the terrestrial compartments is understudied, although majority of the atmospheric mercury is estimated to be deposited in terrestrial environments. As a result, the transfer and bioaccumulation of total mercury (THg) and methylmercury (MeHg), and the associated risks to terrestrial predatory birds and wildlife needs further investigation. This study focuses on bioaccumulation of THg and MeHg in barn owls and their food chain using down feathers of the barn owls, the fur from regurgitated pellets and soil from around the nests. We hypothesize that the source of variation in the barn owl feather concentration are related to the prey species and the diet of the prey species. Furthermore, this study aims to investigate the possible local pollution sources of mercury in Switzerland using the feather, fur, and soil mercury levels.

A direct mercury analyser (DMA-80) was used to quantify THg. A high performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) was used to quantify MeHg.

THg in the down feathers ( $n = 245$ ) ranged between 20 and 1474.8  $\mu\text{g kg}^{-1}$  (average  $\pm$  standard deviation:  $170.5 \pm 156 \mu\text{g kg}^{-1}$ ). MeHg in down feather ( $n = 42$ ) ranged between 34.4 and 503.6  $\mu\text{g kg}^{-1}$  ( $154.5 \pm 99.7 \mu\text{g kg}^{-1}$ ). The MeHg fraction of the THg (% MeHg of THg) was  $83.7 \pm 4\%$  ( $n=42$ ). THg in the fur samples ( $n=127$ ) ranged between 11.2 – 1230.8  $\mu\text{g kg}^{-1}$  ( $165.9 \pm 214.5 \mu\text{g kg}^{-1}$ ). %MeHg of THg was  $56.4 \pm 11.3\%$  ( $n=12$ ) and  $7.3 \pm 4.9\%$  for inorganic Hg fraction. THg in the composite soil samples ranged between 34.9 – 118  $\mu\text{g kg}^{-1}$  ( $63.7 \pm 17.1 \mu\text{g kg}^{-1}$ ;  $n = 64$ ).

The down feathers of barn owl juveniles are good bioindicator of local pollution as they have not fledged out of the nest and the only source of mercury exposure can be related to the diet. The diet is an important source of variation, as the composition (number and species of prey) differ and hence effects the mercury accumulation from the various diet of the prey-species within the food chain. In addition, the mean values for THg in the barn owl feathers and fur of the prey studied are unlikely to cause adverse reproductive or behavioral effects. The possible local pollution sources are understudy. All soil mercury levels were found below the guidance value set by the Swiss Soil Pollution Ordinance for multi-functional land use.

### 3.24 State-Of-The-Art Analytical Tools for an Enhanced Non-target Screening of Environmental Samples

#### 3.24.P-We234 Class Identification of Nitrated Organic Compounds in Ambient Particulate Matters by Using Electron Capture Negative Ionization

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Ambient fine particulate matters ( $\text{PM}_{2.5}$ ) can pose risks of public health, especially in populated urban regions with intense anthropogenic emissions. Nitrated organic components are potential toxic compounds of  $\text{PM}_{2.5}$ , such as organic nitrates (ONs, with a covalently bound  $\text{ONO}_2$  group) and nitrated aromatic compounds (NACs, with  $\text{NO}_2$  group connected to an aromatic ring). However, chemical compositions of nitrated organic compounds remain unknown in urban areas. Leveraging the high electron affinity of  $\text{NO}_2$  group, this study coupled electron capture negative ionization (ECNI) to gas chromatography and time-of-flight mass spectrometry. 78 unknown ONs were screened through their fragmentation patterns from >1300 organic compounds of  $\text{PM}_{2.5}$  in Beijing. 12 compounds were confirmed by self-synthesized reference standards. Precursors of 65 compounds are anthropogenic alkenes. Substituent positions ( $\alpha$  or  $\beta$  carbon) of  $\text{ONO}_2$  group in  $\alpha$ -alkene-generated hydroxynitrates indicate the OH-initiated radical addition reaction in daytime is the dominant formation mechanism. Due to low abundance in  $\text{PM}_{2.5}$ , NACs cannot be comprehensively detected by high-resolution mass spectrometry. The molecular characteristic of high electron affinity and resonance-stable structure makes NACs the only nitrated organic components in  $\text{PM}_{2.5}$  that can yield molecular ions through electron capture ionization, and  $\text{NO}_2^-$  is the only negative product ion after the collision induced dissociation. Therefore, NACs

can be comprehensively detected without the help of reference standards, through the combination of ECNI and gas chromatograph and tandem mass spectrometry. Eventually, 1047 non-polar NACs were detected from PM<sub>2.5</sub> of Beijing. Element compositions of 128 compounds were acquired from the high-resolution mass spectrometry. 25 compounds were confirmed by reference standards. Except for NPAHs and their alkylated congeners, oxygenated NPAHs were identified, indicating nitration products of oxygenated PAHs. The cluster analysis shows there exist diverse secondary formation pathways such as heterogeneous nitration and nitration after oxidation, except for primary emission and gas-phase direct nitration.

### 3.24.P-We246 Discovery of Carboxylic Acid PAH Metabolites in Fish by Ion Mobility Quadrupole Time-of-Flight Mass Spectrometry

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Alkylated polycyclic aromatic hydrocarbons (alkyl-PAHs) can be toxic to marine organisms. Carboxylic acid metabolites from various alkyl-PAHs have been detected in humans, rodents, and marine worms. These metabolites have however not been identified in marine fish. The analysis of PAH metabolites in fish bile is an established biomarker for ongoing and recent PAH exposure. But investigations of the full metabolome of PAHs is challenging due to low availability of reference standards. New and sensitive ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) methodology enables tentative identifications with accurate mass and MS<sup>E</sup> spectral data. An additional dimension in structure information can be provided by a compound specific collision cross section value obtained from ion mobility spectrometry.

This study hypothesized that marine fish produce PAAs as metabolites after exposure to alkyl-PAHs. Atlantic haddock (*Melanogrammus aeglefinus*) were exposed to phenanthrene, 1-methylphenanthrene or 1,4-dimethylphenanthrene via intraperitoneal injection. A Vion Ion Mobility Quadrupole Time-Of-Flight Mass Spectrometer was used to screen for PAAs in extracted bile samples. An in silico library, in addition to filters for common neutral losses and suspected common fragments were included in the data processing workflow. The results show the first report of PAA metabolites in fish, and how they participate in several different metabolic products. Using modern instrumentation and a comprehensive workflow, we present tentative identifications that expand the scientific knowledge about alkyl-PAH metabolism.

### 3.24.P-We248 Quality Prediction of Tandem Mass Spectra of Environmentally Relevant Compounds using Machine Learning

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The acquisition of tandem mass spectra (MS2) combined with the ever growing comprehensiveness of spectral libraries have greatly contributed to the improvement of suspect and nontarget screening analyses. Nevertheless, discrepancies still exist between the number of potentially relevant contaminants present in environmental samples and those for which spectral information is available. Moreover, issues concerning the quality of the information in databases still exist, including insufficiently curated tandem mass spectra. Based on promising results obtained in proteomics and the added value of automatically assessing MS2 quality, this work focused on developing a machine learning pipeline that allows determining the quality of MS2 spectra of environmentally relevant compounds. A dataset of 204 reference standards of environmental contaminants acquired with different collision energies, corresponding to more than 1300 MS2 spectra, was used. Initially, the focus was set on finding relevant and non-redundant features which could be used for machine learning purposes and that provided a sufficiently accurate representation of the raw input data. Specifically, three different feature sets were computed, and their performances were evaluated using a Random Forrest Classifier with cross-validation and filtered to select those which explained most of the available data. Subsequently, the optimised feature sets were evaluated against the test set and a final classification model was optimised to discriminate between MS2 spectra of good and bad quality. Favouring precision, to minimise the risk that BAD spectra would be labelled as good, the final classifier was able to reach a precision of 85%, whilst still having an accuracy and recall of 79% and 65%, respectively. To the best of our knowledge, this is the first time that a machine learning algorithm was developed to automatically assess the quality of tandem mass spectra of environmentally relevant compounds.

### 3.24.T-01 Identification of Emerging Persistent and Mobile Substances by HILIC-Driven Effect-Directed Analysis

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Substances with a certain combination of intrinsic properties pose a risk to drinking water sources. The combination of the two persistent (P) and mobile (M) properties of substances increases the chances of substances passing natural barriers, such as river banks, and artificial barriers in water treatment plants. Consequently, the presence of persistent and mobile (PM) substances in drinking water sources is currently unavoidable and may pose a risk to human health. Non-target screening based on reversed-phase (RP) liquid chromatography high resolution mass spectrometry (HRMS) is being used more frequently to identify emerging compounds. However, unconventional chromatographic approaches should also be considered to analyse PM substances due to their high polarity. Furthermore, identification priority should be given to PM substances that pose a risk to human health, which can be facilitated by the use of bioassays.

This study shows the application of an effect-directed analysis (EDA) method for the analysis of surface and well water samples from the Rhine River. The sample preparation approach used enables a significant preconcentration of a wide range of PM compounds (multi-layered solid phase extraction) and preserves the original composition of the samples (evaporation). The

extracts of the samples were separated using two orthogonal separation techniques - RP and hydrophilic interaction liquid chromatography (HILIC) and analyzed by HRMS. The same extracts were fractionated using high-resolution fractionation collector (FractioMate) to guide chemical identification to the biologically relevant fractions, which greatly reduces the complexity of the identification approach. The prioritization of features for the identification was based on a partly automated transthyretin-binding assay that measures the competitive binding of chemicals to thyroid hormone distributor protein transthyretin (TTR). TTR-binding assay for the RP and HILIC fractionated samples showed active fractions in both separation modes, which facilitated the prioritization of features with human health relevance.

### **3.24.T-02 Suspect and Non-Target Screening of Per- and Polyfluoroalkyl Substances in Electrochemical Oxidation Treated Water using HRMS and in silico Techniques**

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Per- and polyfluoroalkyl substances (PFAS) is an anthropogenic class of substances with unique physical and chemical properties. They have a large variety of uses but are mostly utilized due to their non-stick and lubricating qualities. Although PFAS has proven to be useful in industry and consumer products they have less desirable qualities. These qualities include being persistent, mobile, toxic and bioaccumulative. With new destructive treatment methods for PFAS a need for advanced and in-depth analysis is necessary. Electrochemical oxidation (EO) in a complex matrix may cause the rise of transformation products containing CF<sub>2</sub> containing moieties, all of which should be identified and characterized.<sup>1</sup> In this study High-Resolution Mass Spectrometry (HRMS) will be utilized to perform suspect and non-target screening of PFAS on EO treated samples with the goal of identifying previously unknown compounds.

A previous suspect screening study was performed on samples from the same location, which will serve as a foundation for the non-target screening study on the transformation products. Water samples were collected at a wastewater treatment plant which treats leachate originating from a landfill. Each water sample was treated with foam fractionation, then the foam itself was collapsed and treated with EO. Samples were collected at various time points, of which in this study the time points included were 1 h, 3 h, 5 h, 7 h and 9 h, as well as a sample that was not treated with EO. The initial results of the non-target screening resulted in five compounds that contained at least one fragment which belongs to a perfluorinated tail and did not give a hit as a PFAS in any databases. Half of the compounds have an intensity that is higher after being treated with EO as opposed to no treatment. Some compounds have similar fragmentation patterns to that of other known substances that have been recorded in MS<sup>2</sup> libraries, but contain a different chain length on the perfluorinated tail. Currently, only a very basic in silico techniques, based on molecular formulas and transformations from other treatment studies, have been used to predict transformation products.

Several compounds have been tentatively identified as PFAS transformation products. The in silico predictions of transformation products have proven to be useful already in the initial stages of data evaluation for identifying features that the normal peak picking algorithm missed.

### **3.24.T-03 LC-TIMS-HRMS Combined with Advanced Data Processing Tools - A Powerful 4-D Workflow for the Identification of Biotransformation Products of Zebrafish Exposed to Xenobiotics**

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The impact of xenobiotics in the aquatic ecosystem is evaluated in more depth when the whole xeno-metabolome (xenobiotics and their biotransformation products (bio-TPs)) of aquatic organisms is studied. Therefore, it becomes compulsory to thoroughly study the biotransformation processes of aquatic organisms.

Despite the technological advancements in LC-HRMS, the identification of bio-TPs remains challenging. A major challenge is the vast number of different biotransformation reactions, while some of them are still under investigation. Thus, there is an urgent need for powerful data treatment workflows to identify yet unknown bio-TPs. On the other hand, even well characterized reactions (e.g., hydroxylation) may lead to different isomeric bio-TPs. To address that, it is imperative to establish efficient analytical methodologies, that provide extensive analytical information. Another important challenge in the context of unknowns' identification, is the lack of experimental DDA MS<sup>2</sup> spectra for a great portion of the detected precursors. Therefore, efficient MS<sup>2</sup> data acquisition modes are required.

Recent studies, demonstrate i) the combination of complementary chromatographic modes with HRMS and ii) ion mobility spectrometry coupled to HRMS as promising alternatives for the determination of small molecules in complex matrices. To overcome the aforementioned challenges, we propose the combination of different orthogonal analytical techniques (RPLC/HILIC, TIMS and HRMS) with sophisticated acquisition methods (PASEF), aiming to create the highest amount of analytical evidence. At the same time to develop an efficient suspect and non-target screening workflow that utilize the extensive analytical evidence to its maximum to facilitate the identification of bio-TPs.

The improved utility of LC-TIMS-HRMS in the identification of bio-TPs will be demonstrated via illustrative examples from exposure experiments of zebrafish exposed to different xenobiotics. The added value of the developed analytical workflow will be discussed, in the context of isomers separation, enhanced sensitivity, improved DDA MS<sup>2</sup> spectra coverage (PASEF) and acquisition of cleaner MS and MS<sup>2</sup> spectra through mobility filtering. The use of *in-silico* prediction tools was an integral part of the identification workflow. Examples from the application of suspect and NTS will be demonstrated, highlighting the identification of complex bio-TP structures formed by subsequent biotransformation reactions.

### 3.24.T-04 Nontarget Liquid Chromatography High Resolution Mass Spectrometry and in silico Structural Characterization of Dissolved Organic Matter from Different Water Sources

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Dissolved organic matter (DOM) of aquatic systems, a heterogeneous mixture of compounds resulting from metabolic activity and decay of organisms, represents one of the largest exchangeable carbon reservoirs on the planet and it is involved in numerous biogeochemical cycles. A holistic comprehension of DOM composition is crucial to assess the health status of the ecosystem, but the complexity of its formulation makes DOM analysis arduous.

Characterization of DOM is commonly performed by direct infusion (DI) high resolution mass spectrometry (HRMS), employing electrospray ionization (ESI) operating in negative mode. However, liquid chromatography (LC) separation represents a complementary alternative to DI which can provide new insights. In this study, LC-HRMS was applied in association with novel HRMS nontarget computational tools to achieve a data analysis workflow that allows, for the first time, the structural identification of DOM composition in water matrixes.

Three water samples (sea, river and drinking water), with different expected content of organic compounds, were considered. Samples were solid phase extracted by PPL cartridges adopting an existing protocol. Analysis was performed in triplicate using both DI and LC coupled to an Orbitrap equipped with ESI. Xcalibur software and MFAssignR package in R were used respectively for peak picking and formula annotation of DI data. While MS-DIAL and SIRIUS were used for LC data. Spectra from LC-HRMS were processed in GNPS (Global Natural Products Social Molecular Networking). Here, features were clustered in molecular networks depending on their MSMS spectral similarity and molecular structures were predicted using the *in silico* Network Annotation Propagation (NAP).

Despite the wide range of molecules detected by DI, this method showed significant limitations since it cannot analyse isomers nor obtain structural information. Whereas, LC and MSMS spectra (from data independent acquisition) ensure the separation of compounds and provide more precise molecular formula annotation and molecular structure prediction. The combination of DI and LC resulted in an improvement in DOM pool characterization. Different fractions of organic compounds were observed by the two acquisition methods. Moreover, LC data treatment workflow allowed the prediction of almost 40% of features detected at a molecular structure level. Implementation of this tool will contribute towards a holistic characterization of DOM composition.

### 3.24.P State-Of-The-Art Analytical Tools for an Enhanced Non-target Screening of Environmental Samples

#### 3.24.P-We233 A High-Content Screening Method for Target, Suspect, and Non-Target Analysis of Chemicals in Sediments Using Gas Chromatography-Atmospheric Pressure Chemical Ionization-Ion Mobility Spectrometry

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Chemical databases have documented over one million chemicals with potential hazards to public health. To keep up with the ever-increasing number of new chemicals in society, environmental analytical chemists are moving beyond characterization of single chemical classes towards complex mixtures. These so-called “high-content” screening methods strive to quantify as many substances as possible, while simultaneously collecting non-target data suitable for identification of unknowns. When applied to dated sediment cores, particularly in remote regions, these methods provide valuable insight into long range transport of environmental pollutants. Moreover, temporal information can be used as a prioritization strategy for identification of emerging chemicals, while at the same time assessing impacts of international regulatory and phase-out initiatives. However, most high-content screening work to date focuses on polar substances using liquid chromatography systems. In this study, a new method was developed and validated for simultaneous target, suspect, and non-target analysis of non-polar substances in sediments. The method utilizes accelerated solvent extraction followed by size-exclusion chromatography, with analysis by gas chromatography-atmospheric pressure chemical ionization-cyclic ion mobility-high resolution mass spectrometry (GC-APCI-cIMS). In comparison to widely-applied electron impact ionization, APCI preserves (quasi-) molecular ions, thereby facilitating identification of unknown compounds. In parallel, cIMS facilitates deconvolution of MS2 spectra while offering additional structural information via the collision cross section (CCS). The newly developed analytical method will be supported by a library of four-dimensional metrics, i.e., retention index, CCS, information of MS1 and MS2, using >100 target compounds with Log K<sub>OA</sub> and Log K<sub>OW</sub> ranging 2.69-15.0 and 1.44-17.5, respectively, which is currently under development. Open science databases and *in silico* predication of fragmentation and CCS will facilitate suspect analysis. Liquid chromatography-based prioritization strategies, such as SIRIUS, will be transplanted to interpret unknown compounds. Once completed, the method will be applied to remote Arctic sediments in an effort to assess variations in chemical storage under a changing climate.

#### 3.24.P-We235 Combined Use of GC & LC - QTOFMS for Wide-Scope Screening of Organic Micropollutants in Surface Water and Wastewater from Pasto, Colombia

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The complexity of the aquatic environment scenario, including urban wastewater samples, together with the huge number of hazardous compounds potentially present in the samples, makes the comprehensive characterization of samples an analytical

challenge, particularly in relation to the presence of organic micropollutants (OMPs). The potential of HRMS for wide-scope screening in environmental samples is out of question, making use of target, suspect and non-target approaches. Considering the different physico-chemical characteristics of OMPs, the coupling to both liquid and gas chromatography to HRMS is indispensable. In this work, the combined use of LC and GC both coupled to Quadrupole-Time-of-Flight Mass Spectrometry (QTOF MS) has been applied for screening of surface water and wastewater samples from Pasto (Nariño, Colombia). The aim was to have a preliminary comprehensive overview of the occurrence of OMPs in the Pasto River, focusing the study on pesticides and pharmaceuticals, to assess the impact of agricultural practices and raw urban wastewater on the quality of the river water.

After SPE with Oasis HLB and C18 cartridges, sample extracts were injected by LC- and GC-QTOFMS, respectively. The searching was made by target and suspect approach, using home-made databases containing more than 1500 compounds (LC-QTOF) and around 500 pesticides (GC-QTOF). The (tentative) identification was possible at different confidence levels, as a function of standards availability and accurate-mass information obtained on the molecular ion/protonated molecule and fragments, and after evaluation of whether the potential fragments were consistent with the chemical structure of the compound. Up to 15 pesticides were identified in surface water in sampling points up-river to the town illustrating the impact of agriculture practices, and 14 pharmaceuticals were found down to the town, illustrating the impact of the urban population. Insecticides and fungicides were mostly identified in the sampling point up to the town, while no pharmaceuticals were found at this site. However, several antibiotics and analgesics, among others, were present in wastewater and the river samples collected in the middle and down to the town. Some metabolites, such as carbofuran-3OH and 4-acetylamino antipyrine, were also identified. Future monitoring will be performed by application of target quantitative LC-MS/MS methods for the most relevant compounds identified in the screening.

### **3.24.P-We236 Considerations in Developing a GCMS Accurate Mass Screening Workflow for Environmental Pollutants**

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When performing non targeted workflows, relying entirely on spectral matching versus a large library such as NIST rarely gives the correct answers, especially what the contaminants are at low level compared to the matrix. Accurate Mass on its own helps but is not the entire answer especially when most GCMS libraries are still nominal mass and molecular ion accurate mass data on its own is rarely enough. We have developed an accurate mass library of >1200 of the most likely environmental contaminants containing accurate mass spectra and retention time on a set method that includes mid column backflush for robustness. The workflow looks at the ppm mass accuracy of a given peak, the retention time, number of qualifiers and the deconvoluted spectral hit. Should all four parameters be within the limits set then these compounds are listed as green, and the operator can be more confident that these are present in the sample. Should at least one but not all parameters be met then that component is listed as amber, and some investigation would be required. Isotope ratio information is significant in reviewing compounds as this is shown.

The presentation will also cover the benefits of accurate mass deconvolution for evaluation of components that are not present within the database and show how to append the database to include those found.

The main benefit of this approach to environmental screening is the removal of false positives without any extensive manual evaluation.

### **3.24.P-We237 Expanding Non-target Screening Capabilities for Per- and Polyfluoroalkyl Substances (PFAS) with Ion Mobility Spectrometry**

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Per- and polyfluoroalkyl substances (PFAS) are a class of manmade compounds used in various household and industrial applications. Production of PFAS began in the 1940s, however a majority of legacy PFAS have been phased out of use due to their toxicity and environmental persistence. Manufacturers have therefore shifted to a variety of structurally modified alternatives, leading to the production of over 9000 known PFAS. Moreover, researchers are now tasked with both the quantitative monitoring and discovery of novel PFAS. Non-target screening approaches are often applied to face these problems, however they can be subject to quantitation, separation, and data annotation challenges. We demonstrate the ability of a multidimensional method coupling liquid chromatography, ion mobility spectrometry, and high-resolution mass spectrometry (LC-IMS-HRMS) to overcome many of these non-target screening limitations. By adding IMS to traditional LC-HRMS platforms, it provides additional characterization capabilities such as measuring an analyte's collision cross section (CCS), or gas phase surface area, for feature and unknown identification using database or in-silico matching as well as  $m/z$  versus CCS trend analyses. IMS was also leveraged to enhance fragmentation capabilities via an all-ions data independent acquisition (DIA) fragmentation scheme using ramped collision energies to fragment each PFAS by its size and charge state. Since the IMS drift times of intact precursors and their corresponding fragments align, straightforward annotations of the complex DIA data were possible even in the presence of co-eluting precursors. This capability further aids in discovering new PFAS compared to traditional non-target fragmentation schemes which only use a single collision energy or focus on a set of the most abundant or specific target ions. IMS also facilitated the separation of PFAS from hydrocarbon-based matrix biomolecules such as lipids which co-extract and co-elute with PFAS due to the different molecular sizes. Additionally, IMS filtering of the matrix signals reduced noise and enhanced PFAS quantitative accuracy in complex samples such as plant material and liver tissue. Finally, the ability to separate constitutional isomers not distinguished by LC using the IMS dimension was demonstrated, allowing for enhanced point source fingerprinting due to the isomers not being grouped together as a single compound.

### 3.24.P-We238 Ion Mobility and Cross Collision Section in Non-target Identification of Environmental Samples – A Need For Prediction Tools

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The identification of chemicals in complex mixtures, such as environmental samples is a challenge due to the presence of both natural and anthropogenic compounds, at different level of concentrations. Separation is a key word to be able to detect low concentration chemicals in such mixtures. Ion mobility separation (IMS) has the ability to separate analytes in the gas phase according to their charge, shape and size in a carrier gas as they travel at different velocity in a drift cell. IMS experiments enable the determination of collision cross section (CCS) value that is a unique physicochemical properties of a molecule that can aid structural studies and also facilitates small molecule identification. However, the number of empirical CCS information available is limited, and prediction tools are needed. Prediction models already exist, mainly for metabolomics purposes, and little for environmental samples, where halogenated compounds are present, but not fully supported by existing prediction tools. The aim is to compare several machine learning prediction tools and a stochastic projection Approximation method (Monte Carlo simulations) with a set of 18 chemicals, including pesticides, pharmaceuticals (fenuron, tramadol, 4-Formylaminoantipyrine, trimethoprim, mirtazapine, metoprolol, sulfadimidine, azithromycin, bisoprolol, venlafaxine, clindamycin, climbazole, cycluron, terbutryn, DEET, candesartan, diclofenac and chloroxuron), for we have CCS values (acquired in an Agilent 6560 LC-IM-QTOF instrument). As isomeres can also be difficult to identify based on retention time and MS/MS only, another set of compounds was used for the CCS prediction. This set includes tris(1-chloropropyl) phosphate, tris(2-chloropropyl) phosphate, and tris(3-chloropropyl) phosphate, and tris(1,3-dichloropropyl) phosphate and tris(2,3-dichloropropyl)phosphate. The first results show that within the set of the 18 compounds, 12 compounds show a difference of CCS values (observed/predicted) below 5 %, 4 compounds between 5% and 10% and 2 compounds with a larger error, between 18% and 25% (azithromycin and bisoprolol, respectively) with the Monte Carlo simulations. With the prediction tool (MetCCS predictor), CCS predicted show less than 5% error for 15 compounds (including the bisoprolol), 2 compounds between 5 and 10%, and one compound (azithromycin) above 10 % (14%). Other prediction tools need to be evaluated for environmental samples.

### 3.24.P-We239 Potential of Ion Mobility Separation in Improving GC-HRMS Screening in Environmental and Food Samples

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The coupling of ion mobility separation to high resolution mass spectrometry (IMS-HRMS) is an advanced tool for wide-scope screening of a large number of organic contaminants and residues in different applied fields. IMS-HRMS can be used in combination with Gas Chromatography (GC) with atmospheric pressure chemical ionization (APCI) source allowing for notably improve the reliability of (tentative) identifications in the monitoring of (semi)volatile compounds in complex matrices. First, the soft ionization promoted by APCI source designed for GC preserves the molecular ion and/or protonated molecule in mass spectra enabling an efficient, rapid, and sensitive wide-scope screening. Furthermore, IMS allows separating species of interest from co-eluting matrix interferences and/or resolving isomers based on their charge, shape, and size. The drift time (DT)-derived collision cross section (CCS) is a robust and matrix-independent parameter, comparable between instruments, of great help in the process of (tentative) identification of compounds detected in samples.

Mobility data for 264 GC-amenable compounds have been collected to build a CCS database, which has been applied to complex-matrix samples including fish feed extracts, surface waters, and different fruit and vegetable samples. The homemade library consisted of diverse families of compounds including pesticides, PAHs, PCBs, flame retardants, and emerging contaminants, such as insect repellents, musks, and UV filters among others. The data collected within this database comprises CCS values for molecular ions and/or protonated molecules and in-source fragments.

Selected examples are used to illustrate the benefits of including IMS in GC-APCI-QTOF MS screening methodologies: a) The IMS DT alignment ( $\pm 0.2$ ms) led to much cleaner spectra in comparison with the conventional HRMS spectra increasing the reliability of the analyte identification; b) The possibility to obtain independent fragmentation spectra at High Energy (HE) by using the appropriate IMS DT-alignment for different “precursors” ions (e.g.  $M^{+\bullet}$  and  $[M+H]^+$ ) when CCS differences between both ionized species are significant (e.g.  $> 2\%$ ); c) the inclusion of CCS deviation (e.g.  $< 2\%$ ) into the identification criteria is an extra value to enhance the confidence in the identification; this is particularly interesting in those cases when the mass accuracy criterion (mass error  $< 5$  ppm) is not accomplished for the fragment ion.

### 3.24.P-We240 Prediction of Retention Time and Collision Cross Section for Different Adducts to Improve Non-target and Suspect Screening of Organic Micropollutants

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Ultra-high performance liquid chromatography coupled to ion mobility separation and high-resolution mass spectrometry instruments are very valuable for the screening of emerging contaminants in the aquatic environment over the recent years.

However, when applying suspect or non-target approaches (i.e. when no reference standards are available) there is no information on retention time (RT) and collision cross section (CCS) values to facilitate identification. Thus, findings solely rely on the match between empirical and theoretical masses for (de)protonated molecules as well as fragment ions to result in MS2 stages. However, false positive tentative identifications could occur and candidates could also match the empirical observations. In this sense, *in-silico* prediction tools of RT and CCS can be of great utility to decrease the number of candidates to investigate as well as to gather more confidence in tentative identifications. In this work, Multiple Adaptive Regression Splines (MARS) was evaluated for the prediction of both RT and CCS. MARS prediction models were developed and validated using a database of 477 protonated molecules, 169 deprotonated molecules and 249 sodium adducts. The database was built using a Vion IMS QTOF instrument featuring a travelling wave IMS separation device. Multivariate and univariate models were evaluated showing a better fit for univariate models to the experimental data. The RT model ( $R^2 = 0.855$ ) showed a deviation between predicted and experimental data of  $\pm 2.32$  min (95% confidence intervals). The deviation observed for CCS data of protonated molecules using the  $CCS_H$  model ( $R^2 = 0.966$ ) was  $\pm 4.05$  % with 95% confidence intervals. The  $CCS_H$  model was also tested for the prediction of deprotonated molecules resulting in deviations below  $\pm 5.86$  % for 95% of the cases. Finally, a third model was developed for sodium adducts ( $CCS_{Na}$ ,  $R^2 = 0.954$ ) with a deviation below  $\pm 5.25$  % for 95% of the cases. The developed models have been incorporated in an open access and user-friendly online platform which represents a great advantage for third-party research laboratories for predicting both RT and CCS data. To the best of authors' knowledge, this is the first study developing RT and CCS predictors for the same instrumental system, as well as the first developed model to predict CCS data of sodium adducts.

### 3.24.P-We241 Advancing Towards the Use of Ion Mobility Separation Coupled to High Resolution Mass Spectrometry for the Analysis of Organic Micropollutants in Aquatic Samples

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Ion mobility separation (IMS) coupled to high resolution mass spectrometry (IMS-HRMS) is a powerful tool for target, suspect and non-target analysis of organic micropollutants (OMPs) in complex environmental samples. In brief, IMS separates ionized compounds based on their mobility through an inert gas in the presence of an electric field. This separation mainly depends on the charge, shape, and size of the molecule. Consequently, IMS permits, in theory, the filtering of interfering species, such as isomeric or isobaric compounds. The implementation of IMS during the analysis of complex matrices permits to enhance the spectral quality compared to conventional HRMS configurations. In addition, IMS can provide an extra identification parameter for the confirmation of OMPs by calculating the collision cross section (CCS) value from the molecule's measured mobility. These values are independent from matrix and chromatographic separation. Thus, the development of freely available online databases of CCS values of organic molecules is of utmost importance for a proper establishment of IMS-HRMS as the routine approach for environmental screening analyses. We aim to highlight the main benefits of using IMS-HRMS instrumentation for the analysis of OMPs in complex environmental matrices. In this light, we present different studies towards the establishment of criteria for reporting identification confidence in aquatic samples, the resolution of isomeric/isobaric substances and the reduction of the false positive findings rate. Additionally, an evaluation of the spectral enhancement provided by IMS, as well as the intercomparability of CCS values between different instrumental set-ups, is shown.

### 3.24.P-We242 Semi-quantitative Non-Targeted Analysis for Emerging PFAS

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Emerging chemical contaminants present a challenge for traditional chemical quantification as they lack reference standards; this is particularly relevant for per- and polyfluorinated alkyl substances (PFAS), which have a wide range of structural diversity and essentially zero available chemical reference data for novel species. Semi-quantitative estimates of chemical concentration can be made for such emerging contaminants using surrogate calibration curves based on related chemicals in the event that reference standards are not available. This is particularly useful when performing non-targeted analysis (NTA), where detection of diverse chemical species is a given, and the likelihood of having comprehensive reference data is low. The uncertainty of concentration estimates in NTA is influenced by NTA data processing, the choice of quantitative surrogate(s), and the mathematical models used to predict concentration from observed instrument abundances. Obtaining accurate concentration estimates is a priority, but bounding the accuracy of such estimates is a necessary step for interpreting the results in a risk-based context. We have compared the performance of semi-quantitative non-targeted analysis workflows in providing concentration estimates. Critically, these data processing approaches were selected to provide bounding confidence intervals for prediction. Model assessment was carried out using a set of 16 PFAS with available reference standards, including mass labeled internal standards, and an additional set of emerging PFAS with reference standards, but no widely available commercial source. Comparisons were made for quantitative accuracy between traditional targeted quantitative approaches and non-targeted data collection, as well as the underlying uncertainty. Estimation error and uncertainty were larger, but acceptable, for NTA derived data when reference standards were available for normalization. Semi-quantitative estimation of emerging contaminants using surrogate calibration with legacy compounds exhibited significant uncertainty, but the models were able to successfully bound the uncertainty using bootstrapped confidence intervals from the test set. Curation of the surrogate population used for concentration prediction and confidence bounding increases the accuracy and decreases the uncertainty of predictions, but development of a generalized approach for surrogate curation is ongoing.



### 3.24.P-We243 Simultaneous Electron and Chemical Ionization used for GC- and Real Time – MS Applications for Improved Non-Targeted Environmental Analysis

**Marleen Vetter**, Sonja Klee and Steffen Braekling, TOFWERK AG, Switzerland

A time of flight (TOF) mass spectrometer operating an electron ionization (EI) and a chemical ionization (CI) source simultaneously is presented for non-targeted environmental analysis. The ecTOF offers multiple setup options that provide the opportunity to use gas chromatographic as well as real time sampling. By coupling both ionization sources directly to one single gas chromatograph (GC), simultaneous structural as well as accurate mass molecular ion information is generated. Hence, target and suspect screening analysis as well as effective non-target analysis using GC-MS for environmental samples is improved considerably.

In addition, the ecTOF can employ real time CI-TOFMS sampling, which is well known for direct measurements of complex mixtures during rapid sample changes. The high resolution, accurate mass TOF mass spectrum continuously creates a full mass-range picture of the sample, gaining information that would otherwise be concealed in nominal mass spectrometry. Using the ecTOF, the real time CI-TOFMS measurements can be complemented by simultaneous GC-EI-TOF analysis of the same sample, allowing for effective separation and identification of isomers using divergent EI mass spectra in one single analyzer.

Various experiments will be presented to depict the performance and highlight the advantages of the GC-ecTOF for non-targeted approaches, specifically for environmental contaminants such as pesticides, PCBs and phthalates. The potential of the ecTOF for real time monitoring is highlighted using an example of biomass burning processes, depicting the burning emissions in high time resolution. Parallel GC-EI-TOF measurements show isomer separation and its improved compound identification potential.

### 3.24.P-We244 Improved Monitoring and Non-target Screening of Indoor Air Samples Using TOF-MS Systems

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Many people spend much of their time in indoor environments, for example at work, at home, or travelling in their car. In these indoor environments, thousands of volatile organic compounds (VOCs) may be present and may accumulate. The quality of indoor air is therefore of high importance as it can affect people's health, comfort, and work performance as well as potentially contaminate sensitive working environments. Hence, monitoring of indoor air has become increasingly important.

This presentation will highlight VOC emission measurements using ISO standard methods. These measurements are performed using a conventional quadrupole mass spectrometer (QMS) as well as a newly developed time of flight mass spectrometer operating an electron ionization and a chemical ionization source simultaneously (ecTOF). Both methods are initially assessed according to the ISO standard, where they are shown to fulfil the performance requirements regarding linearity, quantification limits, etc. Furthermore, a non-target approach to tentatively identify as many compounds as possible is employed for both. Here, compound identification of different indoor samples such as flooring or artificial leather using both systems was performed. The comparison showed that up to 31 % of compounds were misidentified using the conventional QMS system as shown by the additional chemical ionization information provided by the ecTOF. Next to increasing identification confidence, the ecTOF was also able to suggest tentative structures or sum formulas for up to 28 % of otherwise unidentified peaks.

In addition, measurements using a real time chemical ionization mass spectrometry (Vocus CI-TOF-MS) to detect VOCs of these materials is discussed. Compounds of interest as identified using the ISO methods were used for monitoring purposes. Due to the high sensitivity of the system, the analysis can be done within seconds, reducing the analysis time considerably. Furthermore, changes in concentration over time can be monitored and owing to the mobility of the Vocus, on-site and online monitoring becomes feasible for environmental monitoring of indoor environments.

In conclusion, this presentation will show the potential of new, state of the art instrumental setups for indoor air monitoring, characterization and non-target screening of environmental samples and their potential to improve upon existing standards and standard measurement technologies.

### 3.24.P-We245 Supramolecular Solvent-Based Extraction Method for the Determination of a Wide Range of Legacy and Emerging Environmental Contaminants in Indoor Dust

**Paula Marcinekova**<sup>1</sup>, Erika Martinelli<sup>2</sup>, Lisa Melymuk<sup>1</sup>, Pernilla Bohlin-Nizzetto<sup>3</sup> and Pawel Rostkowski<sup>3</sup>, (1)Recetox, RECETOX, Masaryk University, Czech Republic, (2)Department of Chemistry "Giacomo Ciamician", Università di Bologna, Italy, (3)Norwegian Institute for Air Research (NILU), Norway

Indoor environments are burdened with synthetic chemicals associated with consumer products, building materials and furnishing, personal care products and pharmaceuticals. These chemicals are detected in both gas and particulate phase of indoor environments and humans are exposed to them constantly. We have a good knowledge of some chemical pollutants that are typically detected in indoor dust; however, less is known about more novel/emerging contaminants such as UV-stabilizers, fragrances, chlorinated paraffins, dechloranes and more. State-of-the-art analytical tools for non-target screening of emerging contaminants are currently exploited in analytical laboratories worldwide and are rapidly developing. However, the methodology to determine multiple chemical classes in single samples is challenging. The objective of our study was to develop an efficient supramolecular solvent (SUPRAS) extraction method suitable for the extraction of a wide range of chemical pollutants in house dust. Ten house dust samples (composite dust) were collected, pooled together, and injected with multiple internal standards including flame retardants, plasticizers, bisphenols, current use pesticides, per- and polyfluorinated compounds, polyaromatic hydrocarbons (PAHs) and substituted PAHs and synthetic musks. Seven different mixtures of SUPRAS were investigated for extraction efficiency for a broad range of chemicals of interest. Liquid chromatography–mass spectrometry and gas

chromatography–mass spectrometry were used for the detection of the targeted chemical classes. Based on calculated recoveries, SUPRAS composed of hexanol, tetrahydrofuran and Milli-Q in 1:2:7 volume ratio displayed the best extracting capability for a broad range of chemicals. The results show recoveries for the more volatile compounds at 40 to 60 % (PAHs, synthetic musks), while the less volatile compounds reach up to 90 % recovery. These recoveries are comparable with recoveries obtained by conventional extraction methods. However, a great advantage of the use of SUPRAS is its ability to extract different polarity compounds simultaneously while keeping a very low SUPRAS to sample ratio. As our preliminary results show, in addition to target analysis, SUPRAS extracts can be used in suspect screening and non-target analysis. This allows for the detection of an even broader range of chemicals, potentially exposing many new and unknown pollutants in the indoor environment.

### 3.24.P-We247 Linking Nucleic Acid Adductome to Environmental Contaminants

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In polluted environments, biological effect assessment is based on biochemical or physiological changes caused by exposure to hazardous substances. However, finding ecologically relevant biomarkers related to chemical exposure is challenging, especially in wild populations, primarily because most biochemical responses are non-specific. In organisms inhabiting polluted environments, reproductive and developmental disorders can be driven by genome modifications, ultimately leading to population decline. DNA adductomics is an emerging -omics technique that includes screening and identifying chemical modifications of DNA. Recent advancements in HRMS analysis provide the scope for precise characterization of such structural modifications, i.e., the DNA adductome.

We propose that these modifications can be used to identify exposure effects at the genome level in biota, thus providing a proactive tool in biological effect monitoring. Using data on environmental contaminants (PAHs and trace metals) from the Baltic Sea sediments, we determined adductome profiles, identified specific adducts as biomarkers of the pollution status, and developed a workflow for environmental diagnostics.

Extracted and digested amphipod DNA was analyzed using LC-HRMS with a Full-MS/Data independent acquisition mode. The data processing employed a graphical user interface MATLAB program *nLossFinder*, available as open source, and based on the common neutral loss 116.0474 Da of deoxyribose. Nearly 100 putative adducts were found in the amphipods, which included markers for epigenetics (N<sup>6</sup>-me-dA), oxidative stress (8-oxo-dG) and many unknown adducts. The DNA adducts were correlated to the contaminant concentrations, and a partial least square regression (PLSR) was used to identify influential adducts for the classification of the contaminated and reference sites. PLSR analysis showed that N<sup>6</sup>-me-dA, the biomarker for epigenetic modifications, was one of the main predictors for classifying sites according to their contamination status.

Structural identification of these unknown adducts can aid in characterizing the effect of contaminants on the genome and searching sensitive biomarkers for environmental health assessment.

### 3.25.A Towards Harmonized Nano- And Microplastics Quantification: Reference Materials, Analytics and Improved Experimental Designs

#### 3.25.A.T-01 Analytical Windows into the World of Microplastics - As Defined by Sampling and Analytical Constraints

**Richard Cross,** UK Centre for Ecology & Hydrology (UKCEH), United Kingdom

Whilst a standard definition of microplastics has yet to be agreed, taking into consideration the definition of a nanomaterial under the recommendation of the European Commission, microplastics may be considered as plastic particles smaller than 5 mm, but greater in size than 100 nm. This presents an analytical challenge for the detection and quantification of microplastics in complex environmental samples that may vary so greatly in size.

No single method can identify and quantify this diverse contaminant across the whole range of polymers, sizes and forms that are encompassed within the “microplastic universe”. Rather complimentary state-of-the-art techniques will be required, each of which has its own specific window into this universe. This “analytical window” is the operational space within the multiple dimensions that can describe microplastic material and must be defined every time we report on microplastic number concentrations from the field. Without defining the analytical window of a study, the longevity of any data produced is severely compromised. This is particularly pertinent for particle number concentrations, where even small shifts in the lower size limit of detection can result in orders of magnitude differences in total microplastic counts as larger plastics fragment into ever smaller particles.

In this presentation, we cover some of the fundamental principles that should be considered when attempting to quantify microplastics in the natural environment, including granularity in data as a function of sampling volume, sensitivity in detection as a function of particle size and quantifying repeatability and minimum QA/QC requirements. We will provide real examples of how to characterise the analytical window that operationally defines microplastics, using a case study of  $\mu$ -FTIR, one of the most commonly employed techniques for chemically specific identification and quantification of microplastics in the environment.

#### 3.25.A.T-02 Systematic Evaluation of Fragmentation of Five Common Synthetic Polymers: Influence of Photolysis, Temperature, and Relative Humidity on Fragment Size Distribution and Material Characteristics

**Katherine Santizo<sup>1</sup>, Patrizia Pfohl<sup>1</sup>, Wendel Wohlleben<sup>1</sup>, Joana Marie Sipe<sup>2</sup>, Mark Wiesner<sup>2</sup>, Sam Harrison<sup>3</sup>, Richard Cross<sup>3</sup>, Gbotemi Adediran<sup>3</sup>, Antonia Praetorius<sup>4</sup>, Brandon Lopez<sup>2</sup> and Claus Svendsen<sup>3</sup>,** (1)BASF SE, Germany, (2)Duke University, USA, (3)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom, (4)University of Amsterdam, Institute for Biodiversity

Stress on plastics from photolysis is commonly evaluated due to its potential to be a leading exposure pathway of plastics throughout their lifetime. Therefore, understanding the dynamics that lead to fragmentation and micro- and nanoplastics release from such exposure is necessary. This study focuses on systematically evaluating the influence of temperature and relative humidity during photolysis exposure on pristine microplastic powders and characterizing the powders at specified intervals. Five commonly used polymers are evaluated: PA-6, TPU, HIPS, LDPE and PP. Three exposure conditions all under ISO4892-2 are used with varying temperatures and relative humidity: (1) 90°C, 28%RH ("Kalahari protocol") (2) 65°C, 50%RH, (3) 65°C, 75%RH. Each polymer is evaluated at three exposure time points: 1000h, 1500h, and 2000h, corresponding to up to two years outdoor (at mid-European latitude). To evaluate the fragmentation, we use an adaptation of the NanoRelease protocol, ISO22293:2020. This allows the quantification of the smallest fragments, below 1 µm, thus allowing the observation of micro- to nano-plastic fragmentation. Material characteristics before and after aging are assessed by ATR-FTIR to measure Carbonyl Index (CI), DSC for crystallinity, and GPC for molar mass. Results indicate that each polymer's fragmentation dynamics varied based on time and exposure condition. For example, the LDPE showed high small particle fragments (< 2 µm) under all exposures. In specific, when exposed to high humidity the increase over time stayed on an upward trend over time. This is a clear distinction from the Kalahari method where the fragmentation values (< 2 µm) stayed relatively similar after 1000 hours with similar fragment counts at 1500 and 2000 hours. In contrast, TPU had steady fragmentation values when exposed to high humidity across all time points and showed more resistance to fragmentation under the Kalahari protocol with significant reduced small particle fragments. In addition, CI results varied with each exposure scenario for all the polymers tested with TPU and HIPS showing large changes in their values. Altogether, our current results show that temperature and relative humidity impact the fragmentation dynamics based on susceptibility of the polymers to each stress and will enable us to parametrize a model of fragmentation in different environmental compartments.

### **3.25.A.T-03 Microplastic Metrology: Creation and Characterization of Microplastic Reference Materials**

**Katherine Shaw<sup>1</sup>, Debra Ellisor<sup>1</sup>, Raleigh Aiden Johnson<sup>2</sup>, Rachel Sandquist<sup>3</sup>, Marquesa Calderon<sup>2</sup>, Meredith Evans Seeley<sup>1</sup>, Rebecca Pugh<sup>1</sup>, John R. Kucklick<sup>1</sup> and Jennifer Lynch<sup>1</sup>,** (1)National Institute of Standards and Technology (NIST), USA (2)Hawaii Pacific University Center for Marine Debris Research, USA (3)HPU Center for Marine Debris Research, USA Micro- and nanoplastics (MNP) are ubiquitous in the environment and have potential health effects on wildlife and humans. Plastic pollution analytical research has grown dramatically in the last 50 years, but methods in microplastic and nanoplastic research lack consistency and standardization. A commonly requested reference material (RM) is microplastic-sized particles of environmentally-relevant polymers and shapes. To meet this need, the National Institute of Standards and Technology (NIST) is creating an RM with multiple micro-size fractions. Furthermore, the lack of environmental RMs with verified microplastic quantities makes it difficult to validate analytical methods. Existing environmentally-collected NIST SRMs have been certified for organic and inorganic chemical pollutants, but until now, have not been examined for microplastics. Several NIST SRMs are good candidates to explore for microplastic certification including sediment, sludge, urban dust, soil, and bivalve tissue. Consumer goods were selected for creation of RMs as they are similar to plastics entering the environment from mismanaged waste and contain relevant plastic additives. Ten kg each of high density polyethylene milk jugs, polypropylene disposable cups, and polystyrene disposable knives were bought from retail manufacturers and cryomilled using a Palla VM-KT Vibrating cryomill at ≤ -150 °C in the NIST Cryogenic Reference Material Production Facility. This presentation will update the community on the status of the NIST RM creation and their many utilities. The second portion of this presentation will cover the potential of two existing NIST SRMs to serve as environmental RMs for microplastics. Two NIST reference materials, SRM 2782 Industrial Sludge and SRM 1941b Organics in Marine Sediment were analyzed for microplastics. An average of  $0.72 \pm 1.15$  microplastics/g was found in SRM 1941b and 0.23 microplastics/gram in SRM 2782 (Figure 1). We expect the plastic and environmental reference materials to become valuable tools to validate and standardize analytical methods to quantify micro- and nanoplastics in the environment.

### **3.25.A.T-04 Granulometric Proxy-Based Geo-Spatial Prediction of Microplastic Inventories in Sediments**

**Kristina Enders<sup>1</sup>, Robin Lenz<sup>2</sup>, Franziska Fischer<sup>2</sup>, Klaus Schwarzer<sup>3</sup>, Guntram Seiß<sup>4</sup>, Dieter Fischer<sup>2</sup> and Matthias Labrenz<sup>5</sup>,** (1)IPF, Germany, (2)Leibniz Institute of Polymer Research, Germany, (3)Kiel University, Germany, (4)Bundesanstalt für Wasserbau, Germany, (5)Leibniz Institut für Ostseeforschung Warnemünde, Germany

Sediments are likely the major storage of the microplastics (MP) legacy in the aquatic environment - being the place of continuous particle deposition. Yet, a reliable quantification of inventories is lacking, primarily for two reasons: data sets are limited and the natural variability in sediments is not accounted for. Key to a reliable quantification of sedimentary compartments is a sound normalisation that accounts for the hydrodynamic regime bias, the natural variability of the energetic conditions by which particles are sorted and distributed. While the lack of harmonisation of lab protocols has been frequently addressed, normalisation procedures of MP as part of data analysis harmonisation are unexplored. Former findings showed a co-occurrence of MP with other natural sedimentary particles and demonstrated their potential as part of normalisation procedures. The major scope of this study is to develop an empirical model approach for an accurate geo-spatial prediction of MP sediment inventories based on such identified granulometric proxies. However, in the investigated Schlei fjord the presence of geomorphological peculiarities, that is a narrowing strait, embayments, and the significant recent and continuous MP input from a WWTP, represent spatio-temporal discontinuities, that appear as deviations in the proxy continuum normalisation curve and challenge a simple prediction based on these proxies only. Parameters that solve the encountered challenges of spatio-temporal discontinuities were developed. Additionally, a mass-based model was set up that bases on particle measurements of all three dimension.

Based on a precursory MP data set (via  $\mu$ -Raman and FTIR spectroscopy) and geospatial, hydrodynamic and sediment proxies generalized linear and tree-based (random forest) models were built to predict over 200 available granulometric proxy stations. Based on nested cross-validation results, the best performing model constellations was determined. Geo-spatial interpolation was applied to calculate the MP inventory.

Using granulometric proxy-based empirical models to quantify MP of entire geomorphological regions based on small-precursory data sets allows realistic assessments of MP inventories across different sedimentary systems. Estuarine environments are especially interesting with regard to anthropogenic land-based input into the aquatic systems and could potentially be used as leading indicators for changing MP inputs to the marine environment.

### **3.25.A.T-05 Microplastic Effects Tests Should use a Standard Heterogeneous Mixture: Multifarious Impacts among Sixteen Benthic Invertebrate Species Detected, Under Ecologically Relevant Conditions**

*Vera de Ruijter<sup>1</sup>, Matthias Hof<sup>1</sup>, Petranta Kotorou<sup>1</sup>, Jesse van Leeuwen<sup>2</sup>, Martine J. van den Heuvel-Greve<sup>2</sup>, Ivo Roessink<sup>3</sup> and Albert A. Koelmans<sup>1</sup>, (1)Wageningen University & Research, Netherlands, (2)Wageningen Marine Research, Netherlands, (3)Wageningen Environmental Research (WUR), Netherlands*

Microplastic is a multidimensional contaminant and requires a risk assessment framework that takes into account all these dimensions, while only considering robust data. Therefore, effect tests should use a diverse, environmentally relevant microplastics (ERMP) standard material and meet high quality requirements. We provide seventeen chronic dose-effect relationships and effect thresholds for sixteen freshwater and marine benthic species exposed to ERMP. ERMP was made from plastic items from nature so that it represented the natural diversity of microplastics, in terms of size, shape and polymer type. The test design fulfilled twenty previously published quality assurance and quality control (QA/QC) criteria. Effect thresholds (EC<sub>10</sub>) were found at ERMP concentrations of  $0.11 \pm 0.17$  (*Gammarus pulex*, growth),  $0.50 \pm 0.37$  (*Lumbriculus variegatus*, growth) and  $1.90 \pm 1.08$  (*Lumbriculus variegatus*, reproduction) % sediment dry weight. A positive effect on survival was found for *Cerastoderma edule* and *Sphaerium corneum* with an EC<sub>10</sub> of  $0.021 \pm 0.027$  % and  $7.67 \pm 3.41$  % sediment dry weight respectively. For the other species tested; *Hyalella azteca*, *Asellus aquaticus*, *Corbicula fluminalis*, *Potamopyrgus antipodarum*, *Tubifex* spp., *Chironomus riparius*, *Alitta Virens*, *Limecola balthica*, *Corophium volutator*, *Arenicola marina*, *Porcellana platycheles*, and *Mytilus edulis*, no effects were detected up to the highest ERMP dose of 10% d.w. This work demonstrates that ecologically relevant effect thresholds can be measured for a contaminant as complex as microplastics, while meeting strict QA/QC criteria. Furthermore, we demonstrate that several lab-based single species effects thresholds of environmentally relevant microplastic particles occur at concentrations lower than those found in the environment.

### **3.25.B Towards Harmonized Nano- And Microplastics Quantification: Reference Materials, Analytics and Improved Experimental Designs**

#### **3.25.B.T-01 Improvements in Micro- and Nanoplastic Mass Detection by TED-GC/MS**

*Paul Eisentraut and Korinna Altmann, Federal Institute for Materials Research and Testing (BAM), Germany*

Micro- and nanoplastics (MNP) remain a hot topic of public interest and an analytical challenge [1]. The thermo-analytical technique Thermo-Extraction-Desorption-Gaschromatography/Massspectrometry (TED-GC/MS) for measuring microplastic mass contents [2] and for estimating tire wear mass contents [3] in environmental samples was presented to the SETAC-community in 2018 [4].

This presentation describes method optimizations that have doubled the measurement speed, reduced limits of detection and allowed the implementation of additional QA measures. The applied optimizations made renewed validations necessary whose results are very promising.

Sample materials are heated in a thermogravimetric analyzer under N<sub>2</sub> atmosphere. The decomposition products are purged from the analyzer using a N<sub>2</sub> gas flow and transferred through a heated coupling device onto a solid phase consisting of polydimethylsiloxane. After the solid phase is loaded with the decomposition products, an auto-sampling robot transports the solid phase to a thermal desorption gas chromatograph mass spectrometer for further analysis. Polymer specific decomposition products allow to conclude on MNP-contents in the sample material. More details can be found in [2,3].

Materials were provided by BAM, BfG, Polymer Source and Sigma Aldrich.

Additional analytical runs performed for QA purposes decrease the overall measurement speed. The standard method protocol was hence improved, reducing the thermal range in which decomposition products are generated and sampled to 200-500 °C, reducing the length of the bakeout step and accelerating the GC-oven program. Finally, overlapping measurements were introduced allowing to perform the following thermal extraction while the GC-run of the former measurement is still in process. For higher comparability between measurements, but also as a measure of quality assurance, inexpensive fivefold-deuterated polystyrene (PS) was used as an Internal standard. However, as some matrices can catalyze an H/D-exchange reaction very effectively [5], <sup>13</sup>C<sub>6</sub>-labelled PS was introduced as a replacement.

In comparison to the former standard method described in [2,3], most characteristics were retained (repeatability) or improved (LoD, linearity).

Besides MNP analysis, polymer aging, analysis of complex composite materials and of interactions between activated carbon and contaminants of pharmaceutical origin represent new research fields in which TED-GC/MS can contribute immensely.

#### **3.25.B.T-02 Identification and Quantification of Microplastic in Environmental Samples by a Combined Method of Separation and Differential Scanning Calorimetry**

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Microplastics in environmental compartments are a topic of serious concern. Time-consuming processes and analytical methods hamper not only the understanding of occurrence and fate of microplastics, but also the derivation of regulatory actions to monitor and minimise inputs in the environment. Therefore, the establishment of a fast, reliable, robust and cost-efficient method for environmental monitoring is required. Most of the common used methods do not fit all requirements. They are highly time-consuming, require high investments or may not give chemical confirmation of the polymer.

We present an approach for microplastic identification and quantification by a combination of separation and differential scanning calorimetry. Experimental data sets for the determination of LOQ for semi-crystalline and amorphous polymers as well as mixtures of polymers in an inert mineral matrix were generated. These data sets were evaluated by different regression methods. In addition to the regression according to DIN 32645, the two-factor regression was also included. The result shows that the two-parameter regression enables a clear identification and robust quantification. The LOQ of the overall process is thereby dependent on the choice of separation method. The method was applied to investigate sediment samples from the entire course of the Elbe River. These examples can be used to illustrate the influence of the separation method on the LOQ, but also the performance and limitations of the method.

### 3.25.B.T-03 The Integration of Further Analysis in a Standardized Biodegradability Test System Can Lead to Better Insights Into Microplastic Degradation

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Microplastics are solid plastic particles smaller than 5 mm. To improve product performance they are intentionally added e.g. in cosmetics, fertilizers, plant protection products, or paints. During application and use they enter the environment in various ways. Potential impacts on environmental and human health have raised concerns, therefore intentionally added microplastics are expected to be restricted by the European Union soon. Exempt from this restriction are biodegradable polymers. The proposed test methods to classify microplastics as biodegradable comprise several standardized test methods in a tiered approach. These proposed methods all target ultimate degradation (mineralization, i.e., organic C is converted to CO<sub>2</sub> or O<sub>2</sub> consumption) as defined biodegradation pass criterion. However, the overall degradation of the polymer is not only the result of the complete mineralization to CO<sub>2</sub> but also other processes such as abiotic degradation (e.g., hydrolysis) or fragmentation (mechanical or biological) and moreover is dependent on several microplastic particle characteristics (shape, size and surface area).

The biodegradation of a polyurea capsule suspension was tested by performing a modified OECD 301B CO<sub>2</sub>-Evolution test. Since the polymeric component of the suspension comprised <1%, <sup>14</sup>C-radiolabeling of the polymer was a pre-requisite to distinguishing the degradation to <sup>14</sup>CO<sub>2</sub> from other components of the suspension. Amongst others, the following modification was introduced: 50 ml aliquots of all replicates were passed through a sequential filtration at day 0, after 28 days and in the end (161 days) with filters consisting of four pore sizes. Thus, polymeric particles were separated by size and quantified by radioactivity measurements. With this approach we found out that the microcapsule suspension mostly consisted of particles larger than 12 µm (54% AR) or particles smaller than 0.2 µm (25% AR). Finally, the mineralization to <sup>14</sup>CO<sub>2</sub> could be attributed to the mineralization of components of all size fractions.

In addition, Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) analysis coupled with a radiodetector was performed to identify the majority of <sup>14</sup>C-labeled molecules of the smallest size fraction, as 8% biodegradation was observed.

### 3.25.B.T-04 On the Potential of Analysing Biodegradability of Microplastics with Stable Isotope Raman Microspectroscopy

**Kara Müller**<sup>1</sup>, Martin Elsner<sup>2</sup> and Natalia P. Ivleva<sup>2</sup>, (1)Technical University of Munich, Chair of Analytical Chemistry and Water Chemistry, Germany, (2)School of Natural Sciences (Dep. Chemistry), Technical University of Munich, Chair of Analytical Chemistry and Water Chemistry, Institute of Water Chemistry, Germany

Biodegradable polymers are designed to reduce environmental plastic pollution, but it is of great importance to show their complete mineralization by microorganisms to avoid assimilation of inert micro- and nanoplastics in soils or waterbodies. Conventional methods either analyze the change of polymer properties during degradation (e.g., mass loss) or microbial activity in terms of O<sub>2</sub> consumption or CO<sub>2</sub> production. However, those methods lack a direct relation between plastic and microbes to see which species are responsible for the degradation and to determine the final fate of the polymers. Therefore, stable isotopes can be used to trace elements from the polymer into biodegradation products. With a Stable Isotope Raman Microspectroscopy (SIRM) approach, we want to monitor <sup>13</sup>C or D fluxes from labeled polymers into single microbial cells or vice versa, based on red-shifted Raman bands of heavier isotopes.

In a direct D-labeling approach, deuterated polylactic acid microplastic particles are incubated with *Sphingomonas koreensis*, which was isolated from an environmental sample and is able to produce carotenoids. Those pigments dominate the Raman spectra due to their resonance effect. Besides those carotenoid spectra, we can obtain full biomass spectra, which show Raman

bands of other cellular compounds after photo-bleaching. Depending on the cellular carotenoid concentration, we use two different strategies to determine the degree of deuteration: 1) CH vibrations are strongly red-shifted from 2990 to 2145  $\text{cm}^{-1}$  (usually Raman silent region) in case of deuteration. Therefore, the peak area ratio  $\text{CD}/(\text{CH}+\text{CD})$  can be used for quantification in the absence of dPLA signals and carotenoid overtones. 2) In the case of strong carotenoid signals ( $\nu_1$ : C=C,  $\nu_2$ : C-C stretching,  $\nu_3$ :  $\text{CH}_3$  rocking), the overtone of their  $\nu_3$  Raman band overlaps with the CD signal and the peak ratio cannot be used. Instead, the difference between the band position of the  $\nu_1$  vibration (red-shifted signal for deuteration), and the  $\nu_2$  vibration (position independent from deuteration) is used to include an internal wavenumber correction.

We also work on a reverse labeling approach, where cells are initially labeled with cheaper  $^{13}\text{C}$ -substrates (e.g., lactate- $^{13}\text{C}_3$ ) and then incubated with non-labeled polymers. Both approaches require statistically relevant reference data to define quantification models, which will be presented in this work together with the applicability of biodegradation experiments.

### 3.25.B.T-05 Differentiating Between Microplastics, Algae and Dissolved Organic Matter Using Single Particle ICP-TOFMS

**Lyndsey Hendriks<sup>1</sup>** and **Denise M Mitrano<sup>2</sup>**, (1)TOFWERK AG, Switzerland, (2)Environmental Systems Science, ETH Zürich, Switzerland

There is a diversity of analytical techniques to measure microplastics (MPs) in complex environmental matrices currently under development, and the selection of which approach to use often depends on the aim(s) of the research question and the experimental design. Researchers around the globe, as well as instrument manufacturers, are working to develop innovative approaches, tools, and technologies to help better characterize MPs and their impact on the environment and human health. Here we further broaden the suite of possible analytical techniques which can directly detect MPs in suspension while differentiating the carbon contained in MPs from other natural particles and dissolved organic matter (DOM). The work presented here makes use of a time-of-flight (TOF) based ICP-MS instrument operated in single-particle (sp) mode to record the signal spikes produced by individual MPs. Spherical polystyrene microspheres of 4  $\mu\text{m}$  were used as model MPs for method validation. These MPs were detected by monitoring the  $^{12}\text{C}$ -content in two different complex natural water systems, namely waters with environmentally relevant DOM concentrations (up to 20  $\text{mg/L}$ ) and in the presence of other carbon containing particles, such as algae cells. The strength of this development lies in the different types of analytical information that sp-ICP-TOFMS can provide from very simple measurements. Indeed, beside providing data on mass concentration (concentration of C per water volume) and particle number concentration (number of particles per water volume), this approach allows to unambiguously distinguish different C-containing species based on their multi-element fingerprint. Consequently, when MPs were suspended together with algae, distinct populations – MPs, single algae cells and heteroaggregates of MPs and algae – could be identified. Furthermore, elevated DOM concentrations did not impact the enumeration of particles in suspension. In summary, the method presented here allows for screening of MPs in aqueous suspensions in natural waters.

### 3.25.P Towards Harmonized Nano- And Microplastics Quantification: Reference Materials, Analytics and Improved Experimental Designs

#### 3.25.P-We249 Quantifying Plastics in the Digestive Tract of Vertebrates: Development of a New Method Applied to Seabirds

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Plastics are now everywhere in our environment. As plastic production continues and waste accumulates, all species are exposed to this pollution to different degrees. Plastic ingestion may have a multitude of direct negative effects, from mortality due to obstruction to reductions in body condition due to nutritional deprivation. In addition, plastic ingestion can lead to adverse toxic effects because of additive chemicals or adhered contaminants that leach from the plastic and are absorbed by the organism. Thus, plastic ingestion can threaten population viability by reducing survival and/or breeding success and consequently, represents a major concern for conservation. Previous studies in vertebrates have mainly focused on macro and large microplastics (items larger than 1mm) and very little is known about the accumulation and impact of smaller microplastics in these animals, limiting our ability to evaluate the long-term impacts of this pollution. This gap is partially due to difficulties in measuring smaller microplastics in animals. To help fill this void, we have adapted a protocol, previously used on invertebrates, to measure macro and microplastics greater than 1.6  $\mu\text{m}$  in seabird tissues using pyrolysis gas chromatography - tandem mass spectrometry. Between 2019 and 2020, 38 yellow-legged gull (*Larus michahellis*) cadavers were opportunistically collected from several colonies in southern France. We evaluated two different methods for treating these samples and quantified both macro and microplastics separately in four different parts of the digestive tube. Our results show high inter-individual variation and differential accumulation of plastics – macro and micro – along the digestive tract. We hope that our method will become a standard that can be used for other vertebrates to better compare plastic ingestion among species and localities and to enable us to better evaluate the toxicity and population risks of accumulated plastics.

#### 3.25.P-We250 Microplastics in Urban Freshwater: A Case Study in the city of Amsterdam

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The contamination of freshwaters with microplastics (MPs) has been globally established. From macro to nano-sizes, plastics enter aquatic ecosystems via direct sources such as discharges from wastewater treatment plants and drainage outlets and indirect sources such as atmospheric deposition, surface runoff, or human activities. In the environment, plastics undergo degradation and weathering processes depending on the physicochemical properties and environmental conditions [1]. Although the presence of MP in freshwater is an indubitable fact, the degree of MP pollution needs further investigation to understand the effects on the ecosystem scale and society. The aim of this study is to determine the MP abundances in the urban region of Amsterdam (The Netherlands) including a large network of canals and waterways, receiving treated wastewater from wastewater treatment plants, and an average annual rainfall of 844 mm. The sampling was done with a state-of-the-art in-situ volume-reduced sampling pump at 11 locations in the Amsterdam canal network in 2022. The qualification and quantification of the polymers for three size fractions (0.7-10  $\mu\text{m}$ , 10-300  $\mu\text{m}$  and 300 $\mu\text{m}$ -5mm) was accomplished in two laboratories at VUA (Amsterdam) and ENPC (Paris) with two complementary analysis methods using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) and micro Fourier transform infrared ( $\mu$ -FTIR) imaging.

### **3.25.P-We251 Microplastics as a Carrier of Selected Heavy Metals from Compost and Its Reduction in Concentration After Effective Separation**

*George Kuttiparichel Varghese<sup>1</sup>, Dilraj S<sup>1</sup> and Christain Zafiu<sup>2</sup>, (1)National Institute of Technology Calicut, India, (2)University of Natural Resources and Life Sciences, Austria*

Microplastics(<5mm) are gaining importance as a carrier of heavy metals into the food webs and across different trophic levels. Compost is considered as one of the major sources of microplastics in soil environment. This study presents the results of analysis for selected heavy metals (Pb, Cu, and Zn) in three samples, i.e., plastic separated compost, hand-picked microplastics from compost and the microplastics separated from compost using chemicals. The compost for the study was collected from the compost plant of Kozhikode Corporation in Kerala, India. The chemical separation of microplastics adopted in this study includes density separation using zinc chloride(1.4–1.5g/cc) and oxidation using Fenton's reagent to remove the organic matter. The average number of microplastics in the compost sample was observed to be 840 items/kg. The three samples were digested using Aquaregia (3HCL:1HNO<sub>3</sub>) and was filtered and diluted for the heavy metal analysis. The concentration of heavy metals in the three samples were analysed using the Flame Atomic Absorption Spectrometer. The results showed a reduction in concentration of heavy metals in the microplastics which are separated using chemicals compared to the hand-picked samples. This reduction in concentration points to the easy transfer of heavy metals to the soil when the compost is introduced in the soil for agriculture. This study shows that the assessment of heavy metals concentrations by the analyses on the microplastics which are chemically separated from compost leads to the underestimation of the ability of microplastics to carry heavy metals from compost to soil.

### **3.25.P-We252 The Release and Toxicity of Microfibers from Single-Use and Reusable Face Masks**

*Yuyue Huang, University of Southern Denmark, Denmark*

The COVID-19 pandemic has caused an unprecedented increase in face mask wearing as recommended by the World Health Organization (WHO) to prevent the spread of coronavirus. The increased production and use of single-use face masks during the pandemic has resulted in a significant increase in mask waste and a large number of used face masks has been found in the environment during and post-pandemic. Single-use face masks can fragment into micro- and nanoplastics which are of concern. Therefore, switching from single-use masks to reusable masks is one of the recommended solutions to reduce plastic waste. However, reusable face masks may also release plastic microfibers during recurrent use. This study aimed to 1) investigate the amount of microfibers released from both single-use and reusable plastic face masks under simulated environmental aqueous conditions, as well as under different washing procedures; 2) chemically characterize the released microfibers using micro-Raman Spectroscopy; 3) evaluate the toxicity of the released microfibers to zebrafish larvae. Up to over 500 microfibers can be released from one single-use face mask. The number of microfibers released from face masks varies largely from different brands and treatments. Hand washing and machine washing treatments release more microfibers than other treatments. Polypropylene fiber is dominant. Given the increasing use and disposal of face masks, the expected increasing release of polypropylene fibers and associated chemicals from face masks into the environment should not be overlooked.

### **3.25.P-We253 Spatiotemporal Distribution of Microplastics in Surface Water and Sediment of Jungnangcheon Tributary**

*Dat THANH Pham and Jung-Hwan Kwon, Korea University, Korea*

Microplastics (MPs) are of great public concerns for the ubiquitous and persistent occurrence in the aquatic environment. Less efforts have been made to monitor the MPs in fresh waters than those in seawaters, although rivers are now recognized to play a vital role in the transportation of MPs, particularly to marine environments. Variations in spatial scale (e.g., river reach, full watershed scale) and temporal scale (e.g., hourly sampling, repeated seasonal samplings) are factors influencing the MPs distribution and abundance. However, much is still unknown regarding their spatiotemporal distributions and links to potential sources. The accumulation or deposition of MPs in aquatic environment is also subjected to hydrodynamic processes. Therefore, the drivers of microplastic fluctuations, including seasonality, the role of resuspension of sediments, as well as the volume of precipitation and runoff, need to be thoroughly addressed. In this study, MPs spatiotemporal distribution in surface water and sediment of Jungnangcheon (South Korea) tributary were investigated using micro-Fourier-transform infrared spectroscopy (micro-FTIR) after appropriate isolation processes. Surface water and sediment samples from five sampling sites along 30 km of the river were collected monthly for a year. The results showed that there was no significant difference of MPs spatial distribution in both surface waters and sediments. Point sources such as sewage effluents may slightly contribute to the higher MPs abundances at some sites. Seasonally, average MPs abundance in water was highest and lowest in summer and winter. The MPs abundance in water in summer was significantly different from other seasons in two sampling sites, however, there was no

significant difference in MPs in sediments between seasons. MPs abundance in water positively and strongly correlated with precipitation at most of the sites. MPs can be trapped in sediment during dry seasons and released to the surface water during wet seasons due to more extreme flows. MPs load was mainly transported by water column in the wet season. The results suggested that it is necessary to consider seasonal factors in analyzing the MPs distribution and transport in surface water.

**3.25.P-We254 Effect of Biomolecules and Environmental Factors on the Aggregation of Colloidal Nanoplastic in Water**  
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Increasing micro- and nanoplastic (MNP) pollution in aquatic ecosystems can have severe consequences on the biota. Biomolecules are essential components of ecosystems, yet they seem to have been overlooked in studies to date. Studying the interaction of MNP with biomolecules is vital to gain better understanding of MNP transport in nature. Chitin is found in arthropods and fungi, and it is the second most abundant biomolecule on Earth, promoting its relevance for MNP-biomolecule interaction in the environment. Chitosan is derived from chitin, and its potential for water treatment and remediation has been studied earlier. However, the interaction of MNP with chitosan is still not well understood. This work explores the interaction of MNP with chitosan in the laboratory under environmentally relevant conditions reflecting fresh/groundwater, brackish water, and seawater. Polystyrene (PS) nanoparticles (NPs) with different surface modifications were used as model MNP and their interactions with chitosan in water at various concentrations, pH, salinity, and dissolved organic matter (DOM) content were studied during batch adsorption experiments. The aim was to identify conditions resulting in aggregation due to interaction between PS and chitosan. DLS-size and zeta potential of NPs were recorded during each experiment. Aggregation occurred only at particular chitosan concentrations, as low as 0.2% (w/w) dose of MNP. At high salinity aggregation occurred independently of chitosan concentrations. Higher chitosan concentrations were required to aggregate NPs at higher pH and DOM content, compared to lower pH and DOM content. The addition of carboxyl groups to the surface of PS to mimic UV photo-oxidation of plastic increased the stability of NPs even at high salinity. However, aggregation still occurred at particular chitosan concentrations. The zeta potential of PS NPs increased with chitosan concentration at low salinity, but aggregation occurred independently of the changes in zeta potential. At high salinity, the zeta potential approached 0 mV, which did not always cause aggregation. Thus, zeta potential alone might not be sufficient to study the interaction of MNP and biomolecules. Overall, this work showed that chitosan can impact MNP transport and has potential for remediation of polluted water.

**3.25.P-We255 A Global Transect of The World's Oceans Monitoring Microplastics - A Citizen Science Collaboration**  
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There have been numerous studies investigating possible effects of microplastics on biota, but what are environmentally realistic concentrations? Estimates of ocean microplastics concentrations are highly variable with offshore sampling stations typically recording much lower concentrations than coastal. Another issue that can complicate risk assessment is that of multiple collection protocols e.g., plankton net trawls versus direct filtration, which introduces high degrees of uncertainty when comparing one area of an ocean to another. An entire global transect of the World's Oceans using the same microplastic collection protocol provides a means of direct comparison and also gives insight into the degree of microplastic contamination in remote areas of the globe. However, such an undertaking would be immensely expensive for a scientific research voyage, but this is where Citizen Science can make a valuable contribution.

In this study, Western Australian Organic and Isotope and Geochemistry Centre (WA-OIGC) researchers at Curtin University collaborated with world-renowned, single-handed yachtsman Jon Sanders to quantify the numbers of microplastic particles present along a global transect starting/finishing in Fremantle, Western Australia, via Mauritius, South Africa, Virgin Islands and Panama Canal, using daily water filtration onto 43 µm aperture stainless steel filters. In particular, the study aimed to provide data for remote areas of the southern hemisphere for which very little data existed previously.

During the 46,100 km circumnavigation, approximately 115 L of seawater was pumped per day from an inlet in the hull, close to the bow of Yacht Perie Banou II. During stopovers in ports, the filters were couriered to the WA-OIGC laboratories for processing and analysed by Attenuated Total Reflectance Fourier-transform infrared spectroscopy (FTIR). The vast majority of the particles observed on the filters proved not to be plastic when analysed by FTIR. However, of the 177 filters collected and analysed, most contained microplastics. A mean count of 33 microplastics m<sup>-3</sup> seawater was recorded across the entire global transect.

The study was the first global transect of microplastics in the oceans that utilized consistent sampling methods throughout. The data was comparable with other scientific surveys of remote areas of ocean and could act as a benchmark for future studies into microplastics in the oceans.

**3.25.P-We256 Reactivity Evaluation between Microplastic Pretreatment Reagents and Ship Paint-Derived Microplastics**  
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As concerns about microplastic pollution in the marine environment are increasing, ship paint-derived microplastics are also gaining more attention. While microplastic pretreatment techniques for removing various organic matters contained in environmental samples are well established, the effects of existing pretreatment reagents on ship paint-derived microplastics, which are likely weaker and more brittle than common microplastics, have not been studied. Since ship paint-derived microplastics exist together with various hull-fouling organisms (e.g. bacteria, microorganisms, small invertebrates, algae,



fish eggs, etc.), it is more important to remove interfering organic matter through appropriate pretreatment technique before qualitative and quantitative evaluations.

Reactivity tests were conducted with three kinds of pretreatment reagents (10% KOH, 30% H<sub>2</sub>O<sub>2</sub>, Fenton's reagent) on nine types of ship paints (two types of primer as anticorrosive paint, two types of tie-coat as adhering agent between the primer and top-coat, and five types of top-coat as antifouling paint and deck paint). Each paint was pulverized into secondary microplastics using a mortar, under liquid nitrogen, and sieved through a 2 mm-sized mesh. After that, they were exposed to the pretreatment reagents for up to 7 days at 40°C and 60 rpm using a thermo shaker. After the exposure to pretreatment reagents, they were vacuum-filtered onto pre-weighed GF/F filter paper and washed with distilled water to stop further reaction. The microplastics on filter papers were dried at 40°C for 24 hours. The pretreated microplastics were compared to untreated control groups in weight and size variations on a microscope, and the changes in physicochemical characteristics were analyzed using Scanning Electron Microscope - Energy Dispersive X-ray Spectroscopy (SEM-EDS) and Fourier Transform Infrared Spectroscopy (FTIR).

For accurate qualitative and quantitative evaluation of ship paint-derived microplastics, the pretreatment reagent should not degrade the microplastic polymer. This study will contribute to more clearly identifying ship paint-derived microplastics by establishing an appropriate treatment method and thus to properly assessing the risk posed by ship-paint derived microplastic sources in the marine environment.

### **3.25.P-We257 Distinctions on Fragmentation and Degradation of Six Common Microplastics from Hydrolysis Condition Variations: Insight into pH, Salinity, and Enzyme Effects**

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It is known that mismanaged plastic waste often ends up in our water bodies (from rivers, lakes to ocean) and enter different stages of the water column from dispersion within the water body, sedimentation to the floors/beds, or floatation on the water surface. It has also been observed that plastic waste in the water bodies is fragmented and/or degraded. However, with the multitude of experimental conditions that can be explored there is a limitation of systematic approaches to comprehend the influence of water conditions on the fragmentation and degradation of polymers. Thus, this study focuses on evaluating the influence of temperature, pH, salinity, and enzymes on pristine microplastic powders and characterizing the powders at specified intervals. Six polymers are evaluated (PA-6, TPU, PLA, PET, LDPE, and PP) in various media (pH 4, pH 9, pH 7, artificial seawater, and enzymatic). The artificial seawater has an additional variation with experiments at 4°C and 22°C, both realistic, complemented by an accelerated aging at 65°C. The OECD Hydrolysis Guideline (OECD Test No. 111) was adapted with time intervals for observation at 5, 30, and 100 days for all but the enzymatic hydrolysis. To characterize the microplastics after hydrolysis multiple analytical methods were used including: an adapted NanoRelease protocol (ISO 22293:2020) to measure below 1 µm fragments, a particle counter providing fragment data from 1-190 µm, ATR-FTIR and GPC for chemical degradation, DSC for crystallinity, and DOC for the dissolved fraction. In addition, pH of the solution and mass of the powder was measured. Preliminary results show that PLA and PA-6 are the most susceptible to hydrolysis both exhibiting larger mass loss than the other polymers. In addition, PA-6 and TPU show larger amounts of particles in the 1-10 µm range after hydrolysis in the high temperature seawater. Furthermore, LDPE under seawater hydrolysis shows no decrease in hydrophobicity while under enzymatic hydrolysis it disperses within the water column showing an increase in hydrophilicity. Although, much of the plastic that enters water bodies is already weathered, this study provides a mechanistic approach to understand the behavior of microplastics under various hydrolysis conditions and will enable us to parametrize a model of fragmentation in different environmental compartments.

### **3.25.P-We258 Interaction Between Microplastics and Polycyclic Aromatic Hydrocarbons Under Sunlight Irradiation**

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The fate of polycyclic aromatic hydrocarbons (PAHs) adsorbed on microplastics (MPs) remains unclear, although PAHs adsorb onto MPs and be transferred into organisms through accidental ingestion of MPs. The main degradation process for PAHs is photolysis, and the rate of photolysis of PAHs and the types of degradation products depend on the materials to which the PAHs are adsorbed. In addition, PAHs, which act as catalysts for photochemical reactions, may enhance the formation of secondary MPs due to degradation of plastic waste under the sunlight. In the present study targeting thirteen PAHs adsorbed with six polymer types of MPs (low-density polyethylene, high-density polyethylene (HDPE), polyethylene terephthalate (PET), polyacrylonitrile, polyvinyl chloride, and polystyrene), the half-lives of the PAHs photolysis ranged from 0.8 (anthracene on PET) to 212.8 (benzo[k]fluoranthene on HDPE) h and were longer than those in the aqueous phase (0.5 (anthracene) – 63.8 (chrysene)) h. Furthermore, we observed that coloration and surface degradation of polyethylene plates adsorbing PAHs under light irradiation, indicating that polyethylene plates adsorbed with PAHs became less tolerant to light. We demonstrate that the interaction between plastics and PAHs results in PAH longevity and accelerated MPs production, exacerbating marine environmental pollution by MPs and PAHs.

### **3.25.P-We259 Ecotoxicity of the Mixture of Microplastics, Nanoplastics, and Additives Produced from the Degradation of Fishing Nets**

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After more than 30 years of research on plastic pollution, data on sources and ecotoxicity are still fragmentary. Several studies have suggested that fishing gear is a major source of secondary micro-nanoplastics (MNPs) and additives. In this context, we focused our research on the ecotoxicity of leachates from the degradation of plastic fishing nets (FNs) during their life cycle, especially those used in the southeastern Bay of Biscay. For this, three gillnets made of polyamide (PA), one in-development gillnet made of bio-sourced and biodegradable polybutylene succinate (PBS), and one polyethylene (PE) trawl net were selected, both new and used ones. First, an artificial aging (AA) protocol based on UV irradiation and agitation was set up to characterize the degradation kinetics of FNs and quantify the released MNPs and additives. The ecotoxicity of leachates was investigated using the Microtox® (marine bacteria *Aliivibrio fischeri*), and fish larval behavior (freshwater medaka *Oryzias latipes*) bioassays. AA caused structural and morphological modifications of the polymers, revealed by FTIR-ATR, RAMAN, and SEM, and induced the release of MNPs into the water, quantified by Morphologically-Directed Raman Spectroscopy (MDRS). Contrary to what was expected, the produced particles were not only fibers but were mainly micro fragments below 100 µm. The degradation kinetics differed among FNs depending on the polymers. Results of the Microtox® and fish larval behavior bioassays revealed significant toxicity of some FNs' DMSO extracts. Toxicity depended on the polymeric matrix, the additives' composition, and the FNs' wear. These results raise concern about the toxicity of new ready-for-use bio-based FNs and highlights the possible major role of additives in the toxicity of plastic FNs. Experiments are underway to understand better these FNs' overall contribution to marine plastic pollution.

### 3.25.P-We260 Micro and Nanoplastics Detection in Small Living Organisms

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Nowadays, micro- and nanoplastic particles are found in a variety of environmental compartments. These can be taken by small organisms at the base of the food chain. Taking advantage of this behaviour, recent studies proposed these as bio-indicators of environmental plastic pollution. However, the application of conventional analytical methods on biological samples show many limitations. As an example, physical-chemical techniques based on pyrolysis have high detection limits, which could hide the amount of plastic collected by such accumulators. On the other hand, optical methods with chemical sensitivity, such as IR or Raman spectroscopy, allow detecting only larger particles or agglomeration of smaller ones. Moreover, a typical analysis usually requires a considerable amount of time. In this project, we are developing a new technique for detecting micro- and nanoplastics in small living organisms based on Hyper-Spectral Enhanced Dark Field microscopy. The setup microscope features a Cytoviva condenser enabling nanoparticle detection, and a wavelength selective optical filter for the hyper-spectral analysis of the scattered light, pixel by pixel. The acquired scattering spectra are then processed by a novel algorithm, based on the Mie model for scattering, which aims at identifying plastic particles from the sample background. This new method, tested onto phyto- and zooplankton species exposed to micro- and nanoplastics, features rapid sample analysis and sensitivity down to the single particle. Significantly, it results particularly effective in detecting particles with size between 100 nanometres and few microns, enabling additionally live particle tracking inside the microorganisms. Finally, it results cost-effective and convenient to be deployed in laboratories involved in plastic pollution research.

### 3.25.P-We261 Multi-Parameter Analysis of Nanoplastics: Taking Advantage of High Time Resolution Enabled by Stimulated Raman Scattering

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The characterization of nanoplastics, especially with regards to their toxicity, is still quite challenging since most analytical techniques can only deliver limited information on this complex analyte. Many different properties, like size, shape, concentration, and chemical composition, have to be considered for a proper characterization and potential risk assessment. To achieve this, it is necessary to use many different (hyphenated) techniques. A technique that can deliver multiple parameters - mainly size and chemical information - from one measurement is online-coupled field flow fractionation (FFF) – Raman microspectroscopy. However, this hyphenated technique still has some limitations, e.g., low sensitivity and dependency on optical trapping, and cannot deliver particle concentrations. For this reason, a coherent Raman technique, called stimulated Raman scattering (SRS), was tested for its potential hyphenation with FFF. This technique employs two different laser wavelengths. Their difference in frequency must match a vibrational transition of the analyte molecule to result in an enhanced signal. Thus, compared to spontaneous Raman, measurement times can be significantly reduced from 10 s to 60.5 µs. The detection of nanoplastics in suspension was achieved directly within the applied flow using a flow cell with either a reflective or a transparent base. Nanoplastics (PS, PE, PMMA) in a size range from <100 nm to 5 µm of spherical and irregular shape could be detected with this setup. In particular, due to the increased time resolution, individual signals per particle could be observed with this technique rather than a average sum signal for spontaneous Raman. Therefore, this method can be used to count particles and also (semi)quantify nanoplastics while also giving information on the material. For the (semi-)quantification a test calibration ranging from 42 mg/L to 500 mg/L was performed on different samples. A closer look at the peak shape of the individual signals reveals that not all detected particles were trapped in the focus of the laser. In case of untrapped particles the mean peak intensity and width can be used for size estimation. Overall, with this method (especially in hyphenation with FFF) a broad characterization of

nanoplastics is possible since information on size, concentration and chemical composition can be obtained within one measurement.

### **3.25.P-We262 Raman and Surface Enhanced Raman Spectroscopy to Detect Submicron- and Nanoplastics**

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The detection of submicron- (100 nm – 1 µm) and nanoplastics (< 100 nm) is of increasing interest within various scientific communities. While the particles are anticipated to be present within many different samples of interest (e.g. environmental systems such as rivers and oceans, or in beverages such as bottled water), their low estimated concentrations, small size, and organic composition make optimizing analytical techniques to study them in these sample matrices a challenging task. To that end, the work presented in this study focuses on the optimization of Raman Spectroscopy for the detection of submicron- and nanoplastic particles composed of polystyrene and poly(ethylene terephthalate). Traditional Raman Spectroscopy measurements were conducted to determine the limit of detection for the standard technique, and then Surface Enhanced Raman Scattering (SERS) substrates composed of gold nanoparticles were employed to improve upon the detection limits determined for Raman Spectroscopy alone. As a further proof of concept, these particles were spiked into true environmental fresh and saltwater collected from seas and rivers. Preliminary results indicate differences in analytical performance when nanoplastics are spiked into freshwater compared to nanoplastics that are spiked into saltwater. Furthermore, the size of the plastic particles as well as the type of plastic they are made of is shown to impact the limits of detection observed.

### **3.25.P-We263 Rapid Detection and Quantification of Micro-plastic Particles using MuScan® Technology**

**Michel Klerks**, *Innosieve Diagnostics BV, Netherlands*

Current simple methods for micro-plastic detection often rely on manually viewing and counting micro-plastics in a sample under a microscope. More advanced techniques use Fourier-transform infrared spectroscopy (FT-IR), Raman spectrometry, and chromatography /mass spectrometry (GC/MS, ICP-MS). However, with these methods a reliable, objective quantification and determination of the size of micro-plastics in a larger sample volume is not possible or very cumbersome. As these advanced methods require large financial investments, need a high degree of expertise and are not easy to perform on-site, there is an urgent need for a more rapid, simple, and cost-effective methods.

We present the development of a fast on-site Solid Phase Cytometry-based method (the MuScan® method) that automatically analyses the number of micro-plastic particles (MNP) in a sample within one hour. The Muscan® method reliably detects, quantifies, and identifies micro-plastics, even down to 1 µm in size.

First a sample is filtered over an inert, optically flat silicon nitride microsieve membrane, herewith retaining all MNPs. Next the MNPs are stained using a fluorescent dye and subsequently the membrane is scanned using an automated LED-based optical scanning device, the MuScan. The total scanning time is only 3 minutes, during which an integrated data processing algorithm runs in the background. The raw data of each pixel area is analysed based on pre-set parameters. Based on fluorescent emission variations between different types of MNPs, a set of different MNPs can be identified. A summary report with counts and size distribution is achieved.

Using this methodology we evaluated different matrices like drinking water and packaged meat for occurrence of MNPs. The preliminary results from these studies will be presented.

### **3.25.P-We264 Rapid, automated analysis of microplastics direct on filters using laser direct infrared imaging and spectroscopy.**

**Darren Robey<sup>1</sup>**, **Wesam Alwan<sup>1</sup>** and **David Troiani<sup>2</sup>**, (1)Agilent Technologies, Australia, (2)Agilent Technologies

The presence of microplastics in the environment is gaining significant public interest. To study this, rapid, reliable, and harmonized characterization is essential. Technical hurdles in microplastics analysis stem from the number of particles to be analyzed in each sample which may range from few to many thousands. While visual microscopy has been used extensively, it may be prone to operator error and bias and is limited to larger particles. Vibrational spectroscopic techniques such as Raman and FTIR microscopy provide an alternative, however analysis time can be significant and more rapid and highly automated techniques are desirable.

The quantum cascade laser (QCL), is an alternative infra-red (IR) source that may assist to address this. In contrast to the FTIR approach, a QCL emits IR energy at specific and discrete wavelengths in a collimated beam with all the energy available and can quickly sweep the wavelength range for sub-second acquisition of spectra. Emitting at individual wavelengths, the QCL source can be combined with a single-point detector and rapid scanning optics allowing very fast discrete frequency scanning of large areas. Combined in this way, very fast, highly-automated workflows can be achieved.

Multifaceted sample preparation is a requirement of all spectroscopic techniques and filtration commonly forms the final step. Conducting microplastics analysis directly on this filter has become a common practice, however this has not previously been possible in the QCL system described as it relies on the use of an IR reflective substrate to perform a “transflection” (double pass transmission) measurement.

The aim of this study was to demonstrate that a suitable filter could function as an IR reflective substrate to achieve both adequate detection of particles on the surface and to obtain spectra suitable for spectral library matching. Achieving this goal eliminates additional sample preparation post filtration which itself reduces the potential for error from poor recovery or sample contamination. At the same time, the increased speed of analysis allows a greater number of samples being processed in a study.

### **3.25.P-We265 Micro- and Nanoplastic Particle Number and Mass-based Analysis on a Plastic-free Size-selective Two Stage Si Membrane Filtration Cascade**

**Paul-Tiberiu Miclea<sup>1</sup>, Korinna Altmann<sup>2</sup>, Yosri Wiesner<sup>2</sup> and Christian Hagendorff<sup>3</sup>, (1)Fraunhofer IMWS - Institute for Microstructure of Materials and Systems, Germany, (2)Federal Institute for Materials Research and Testing (BAM), Germany, (3)Fraunhofer CSP - Center for Silicon Photovoltaics, Germany**

A quantitative metrology and testing of microplastics requires representative methods for sampling, sample preparation and detection for all size ranges of micro- and nanoplastics (MNP). For this purpose, plastic-free filter materials have to be configured to allow a size selective analysis of MNP particles with different polymer types and integrate those into the analytical workflows. Analytical routines for particle sizes in the range of 10 - 1000  $\mu\text{m}$  are nowadays quite well established. Current scientific efforts are focused on entering nano particle dimensions with diameters down to 10 nm. A major challenge lies in the availability of cascade filter setups spanning several orders of magnitude in a single filtration workflow with appropriate MNP analytic routines for both, particle and mass-oriented investigations.

In this work, we are implementing a well-defined microplastics test material for the development of a two-stage cascade filtration setup and subsequent application in MNP analytic workflow. The MNP test material is composed of a mixture of a polydisperse PET microplastic material mimicking environmentally particles and monodisperse PS spheres. The novel cascade filter system contains two filters made of microporous Si, thus realizing the required MNP size selectivity. In our experiments, we demonstrate the MNP size separation on Si filter substrates. Using the filters subsequently as analytical substrates, leads to an enhanced flexibility in the application of  $\mu$ -Raman, scanning electron microscopy (SEM), atomic force microscopy (AFM) and even thermal extraction/desorption-gas chromatography/mass spectrometry (TED-GC/MS) instrumentations. We are able to analyse MNP properties, e.g. particle shape, size, spectroscopic fingerprints and mass - optical and chemical, both qualitatively and quantitatively afterwards on the same sample and filter in direct correlation.

Finally, requirements and limitations for a standardized plastics-free MNP filtration and analytic workflow will be discussed.

### **3.25.P-We266 Real-life Airborne Micro- and Nano-plastic Particles and Fibres on a Porous Membrane Filter System from a Textile Factory Suitable For Both: Vibrational and Mass Spectrometry**

**Paul-Tiberiu Miclea<sup>1</sup>, Korinna Altmann<sup>2</sup>, Yosri Wiesner<sup>2</sup> and Emilia Visileanu<sup>3</sup>, (1)Fraunhofer IMWS - Institute for Microstructure of Materials and Systems, Germany, (2)Federal Institute for Materials Research and Testing (BAM), Germany, (3)National Research and Development Institute for Textiles and Leather, Romania**

Quantitative metrology and testing of microplastic particles and fibres require a good representative method for sampling, sample preparation, and detection for all nano- and microplastics (NMP) size ranges. For this purpose, filter materials must be configured to allow a size-selective analysis of NMP particles and polymer types under ideally plastic-free conditions to be included in analytical workflows.

Analytical routes for microplastic particle sizes in the range of 10 to 1000  $\mu\text{m}$  [1] are nowadays quite well established. Current scientific efforts are focused on entering nanoparticle dimensions under plastic-free conditions with diameters down to 10 nm. A major challenge lies in a suitable substrate for a combination of vibrational and mass spectrometry detection methods and the application of both methods on the same system.

In this work, we are implementing a real-life airborne NMP test material for the filtration workflow and subsequent NMP analytics. The NMP test material is collected in different textile factories. Using the filters subsequently as analytical substrates lead to enhanced flexibility in the application of  $\mu$ -Raman, scanning electron microscopy (SEM), and atomic force microscopy (AFM) instrumentation. We are able to analyse NMP properties, e.g. particle shape, size, and spectroscopic fingerprints - optical and chemical, both qualitatively and quantitatively. Thermo extraction/desorption-gas chromatography/mass spectrometry (TED-GC/MS) can also be applied to the same filters and is used to identify the polymer types and quantify the mass of the material collected. Finally, requirements and limitations for a standardized filtration and vibrational and mass spectrometry workflow will be discussed.

### **3.25.P-We267 Advancing a Semi-Automated Polarized Light-Nile Red Method for Identifying Microplastics in Environmental Samples: From Proof-of-Concept to Protocol**

**Christine M Knauss<sup>1</sup>, Elizabeth North<sup>1</sup> and Christine Thompson<sup>2</sup>, (1)University of Maryland Center for Environmental Science, USA (2)Stockton University**

Microplastic pollution can have harmful effects on the environment and human health depending on the type of plastic, particle size and shape, and exposure concentrations. However, the diversity of microplastics and associated particles in the environment makes the detection, identification, and quantification challenging and current methods to accurately detect and identify microplastics are time consuming and expensive. Because of these challenges, large data gaps exist for the fate, transport, and risks of microplastics. An accurate, fast, and low-cost method is needed to characterize microplastics in environmental samples to comprehensively understand microplastic abundance, fate and transport, and possible risks. One innovative method, combining polarized light microscopy with a semi-automated image capture system and fluorescent light microscopy, is inexpensive and can accurately detect a variety of microplastics with more reliability than either method individually. The objectives of this study are to set up a system with polarized and fluorescent light and a semi-automated image capture system on the same microscope, then develop a protocol to analyze environmental samples with this system. Known microplastics and unknown microparticles will be used to develop the detailed protocol for sample preparation, image capture, and image analysis, culminating in particle identification. Raman spectroscopy will be used to validate classification of samples processed using the new protocol. An overview of the protocol and preliminary data will be presented and discussed. If successful, this inexpensive, and high

throughput method for bulk sample analysis will decrease the resources needed to analyze environmental samples and thus promote better understanding of microplastic presence, fate, and transport in different environments.

### **3.25.P-We268 The How to Guide: An Easy and Accessible Way to Calibrate Fluorescence Microscopes and Modify Raman Spectroscopes for Use with Nile Red Stained Microplastics**

**Anna Tuulikki Kukkola**, Liam Kelleher, Iseult Lynch, Greg Sambrook-Smith and Stefan Krause, *University of Birmingham, United Kingdom*

In the field of microplastic (MP) research, there have been calls for reproducible and universally accessible and inexpensive methods, with low false positive and negative counts. The use of the Nile Red staining method has been put forward as a candidate, due to its low-cost and accessibility. However, not everything that fluoresces when stained with Nile Red should be counted as MP and setting up fluorescence of detection limits is imperative. We trialed several different microscope settings with known plastic types ( $n=13$ ), as well as some common natural materials (e.g., sand, leaves etc.;  $n=5$ ) for establishment of the fluorescence detection limits. Magnification and light intensities had different effects on distinct plastics and no clear trend was evident. By the usage of descriptive statistics, we identified the lowest pixel brightness cut-off limit whereby over 97% of plastics were detected and only 22% of rare false positives. Following the steps presented herein, fluorescence systems can be calibrated to increase comparability and transparency in microplastic research.

We also present guidelines for a cost-effective and simple modification to existing Raman spectroscopy systems to enable use in combination with the Nile Red staining in fluorescence mode to overcome the issue of potential false negatives as well. The false negatives are non-fluorescent particles unintentionally moved with suspected fluorescent MP onto a new filter when a sub-sample of suspected MPs are intended to be analysed with Raman spectroscopy or similar, when best-practise guidelines are followed. These particles are impossible to tell apart from the targeted fluorescent particles under bright-field but via Raman can be identified as false-negatives.

The modifications took around 3 hours to complete on an existing Renishaw rm2000 system and the parts required cost £1,729 at time of purchase (2022, from Thorlabs). After installation, the plastic standards were visualized in the fluorescence mode, and a strong fluorescence signal from the plastics was detected, suggesting that with these relatively cheap modifications, it is possible to guide identification of MPs that can then be measured with Raman spectroscopy for polymer identification.

These modifications to the standard Nile Red staining protocol (establishment of pixel brightness limits and Raman for identification) will facilitate increased reproducibility, transparency, and simplicity of MP research.

### **3.25.P-We269 Automated Representative Quantitative Analysis of Microplastics down to 1 $\mu\text{m}$ by Raman Microspectroscopy**

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Microplastics (MPs, synthetic polymer fragments in the size range 1  $\mu\text{m}$  – 1 mm) are found in the environment all around the globe as well as in drinking water and food. Since more hazardous effects are expected from smaller MPs, reliable quantitative analysis is required. Here, Raman microspectroscopy (RM) is suitable for the chemical identification and quantification of MPs down to 1  $\mu\text{m}$  ([doi.org/10.1021/acs.chemrev.1c00178](https://doi.org/10.1021/acs.chemrev.1c00178)).

To ensure that the results are reliable and representative, a (very) high number of particles/fibers has to be analyzed, especially for small MPs. Therefore, automation of RM analysis becomes essential. Our open-source program *TUM-ParticleTyper* ([doi.org/10.1371/journal.pone.0234766](https://doi.org/10.1371/journal.pone.0234766)) enables the automated detection, quantification, and morphological characterization of (plastic) fragments in images of optical microscopy, followed by the automated RM-based identification of MPs and non-plastic fragments. This approach has been already successfully applied for the analysis of up to 7000 particles/fibers down to 10  $\mu\text{m}$ , randomly selected on the entire filter ([doi.org/10.1016/j.watres.2022.118549](https://doi.org/10.1016/j.watres.2022.118549)).

Since the number of particles (exponentially) increases with decreasing the particle size, it becomes nearly impossible to detect all particles down to 1  $\mu\text{m}$  on the entire filter. Therefore, we proposed an alternative strategy – random window subsampling, where the automated acquisition of optical image and localization of (MP) fragments are followed by RM measurements from window to window. We also introduced a bootstrap method, to provide an error quantification with confidence intervals from the available window data. Ultimately, we developed and implemented in *TUM-ParticleTyper 2* a new measurement algorithm that computes confidence intervals *on-the-fly* during the analysis using bootstrap and, by checking whether given precision requirements are already met, automatically stops if an appropriate number of fragments is identified, thus improving time efficiency ([doi.org/10.1007/s00216-021-03326-3](https://doi.org/10.1007/s00216-021-03326-3)). Furthermore, we implemented advanced image processing for better recognition and morphological characterization of small (MP) particles and fibers. In this presentation we will discuss our open-source program *TUM-ParticleTyper 2*, which enables automated representative analysis of MPs in the entire size range, with the focus on the program validation and applicability for the analysis of reference MPs ([doi:10.3389/fchem.2020.00169](https://doi.org/10.3389/fchem.2020.00169)) and environmental samples.

### **3.25.P-We270 Electrostatic Separation for a Comprehensive Microplastic Monitoring in River Sediments**

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There are still no standardized processes, to address a comprehensive microplastic (MP) monitoring in real environmental scenarios. Such methods must process a high number of samples and large sample volumes time- and cost-efficiently. Especially for sediment samples, current methods lack representative sample volumes and masses. We want to introduce electrostatic separation (ES) as a method to overcome this problem. We applied ES to different particulate matrices such as quartz sand and various river sediments. Thus, we were able to derive a feasible working range of this method. Moreover, a comparison of recovery rates for different sample sizes, MP types and, contents was carried out. Nine different particulate matrices were used to investigate the particle size dependency of ES. Quartz sand samples (Cemex AG, Dresden, Germany) with a mass of 100 g and 1000 g were spiked with 75 mg of MP low-density polyethylene (LD-PE), polyethylene terephthalate (PET) and polycaprolactone (PCL; total MP mass per sample: 225 mg) in a size range of 63-200  $\mu\text{m}$ . Additionally, 1000 g of quartz sand were spiked with different contents of LD-PE. MP quantification was carried out by differential scanning calorimetry (DSC). Our results show a great potential of ES to enrich samples of quartz sand with an average particle size above 200  $\mu\text{m}$ . Even small contents of finer particles seem not to have an influence on the separation result. MP was successfully separated from quartz sand by ES. The recoveries are in a moderate range for all MP types (50 to 100 %). In the range of 10 to 80 mg LD-PE per kilogram sand no significant differences of the recovery were found. The average recovery was  $90 \pm 8\%$ . The theoretical limit of quantification was determined as 2.3 mg MP in 1 kg sediment. We conclude that ES can facilitate a fast and reliable extraction of MP from particulate matrices. The application of ES and DSC for MP analysis in river sediments shows a very high potential to address comprehensive monitoring time- and cost-efficiently. However, further research is required to address smaller particle sizes and more polymer types.

### 3.25.P-We271 Optimising $\mu\text{FTIR}$ Analysis Measurements Time and Quality with Artificial Intelligence

*Thibault Schowing, IICT, HEIG-VD, Switzerland*

Today, microplastics are ubiquitous and can be found from the highest mountains to human embryo. These plastic microparticles are present but their quantity, sizes and composition remain poorly charted. FTIR (Fourier Transform InfraRed) microspectroscopy is among the non-destructive procedures allowing to characterize both aspect and composition of these particles using the advantages of microscopy imaging and spectrum identification. The characterization of microplastics with  $\mu\text{FTIR}$  microscopy involves, as a first stage, detecting and selecting particles from microscopic images for which an infrared absorption spectrum will be further measured and computed. This step is currently performed with the assistance of a proprietary, instrument built-in tool that shows low flexibility and biased performance on a quantitative and qualitative point of view. Using Artificial Intelligence (AI), more exactly machine learning, to accelerate this step has entailed two main challenges. First, the proposed AI solution must have the ability to interact tightly with the instrument to process incoming images and to highlight in it a set of candidate particles. To address this challenge, we adopted GEPARD, a publicly-available software developed for a subset of Raman and  $\mu\text{FTIR}$  instruments.

The second challenge is the amount of high-quality annotated data that is required to satisfactorily train AI-based detection models. We have addressed this by means of a semi-supervised learning approach. Starting with a few available unannotated images and using data-augmentation techniques to feed an available industry level freeware for the annotation of microscopic images, we were able to create a synthetic ground truth of thousands of annotated images, with each particle clearly labelled on each image. On the base of such annotated data, we were then able to train an AI-based predictive model, compatible with GEPARD, allowing us to rapidly detect particles on the instrument images, potentially saving precious time with respect to the current process.

### 3.25.P-We272 Harmonizing and Expanding Py-GC/MS for the Analysis of Environmental Plastics

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The chemical analysis of micro and nanoplastics in complex matrices is a priority for human and environmental health research. Spectroscopic techniques (e.g., Fourier transform infrared or Raman spectroscopy) are commonly employed to identify a particle's polymer type, but require size and density approximations to estimate mass-based concentration. Mass-based estimates of, for example, body burden are increasingly requested for environmental risk assessment. Further, spectroscopic techniques require extensive sample preparation to isolate individual particles and are not applicable to particles below 1  $\mu\text{m}$ , limiting sample throughput and analysis of nanoplastics. Pyrolysis – gas chromatography/mass spectrometry (Py-GC/MS) is uniquely suitable for the mass-based analysis of plastics as it employs high-temperature thermal degradation of large polymers to generate GC/MS amenable marker compounds (often, a monomer or the polymer). Researchers have used such marker pyrolysates to identify polymer type, as well as quantify polymer concentration using external standards. Uniquely, Py-GC/MS may also detect additive content at low (i.e., thermal desorption) temperatures, but little work has demonstrated the ability to quantify additives in plastics and the polymer simultaneously. We reviewed the application of Py-GC/MS for analysis of micro and nanoplastics in environmental samples, ultimately synthesizing recommendations for future harmonization and possible avenues for method expansion. This begins with sample and calibrant preparation, including which type(s) of preparation are required for different matrices and how they affect quantitation, including a synopsis of limits of detection reachable using these different approaches. The unique requirements for emerging nanoplastics work are highlighted. Toward improving quantification, recommendations for isotopically-labeled standards are included. The merits and pitfalls of different Py-GC/MS thermal programs (e.g., flash or double-shot) are highlighted, including their capacity to qualify polymer additive content. Further, aspects of Py-GC/MS that have not been previously employed for the analysis of environmental plastics will be proposed (e.g., semi-quantification of oxidation in weathered plastics). In total, this poster will open the floor to discuss the timely harmonization and improvement of Py-GC/MS approaches in the environmental plastics field.

### 3.25.P-We273 Establishing Spectral Library of Ship Paint-Derived Microplastics by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope - Energy Dispersive X-ray Spectroscopy (SEM-EDS)

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Although the International Union for Conservation of Nature (IUCN) and the International Maritime Organization (IMO) identified marine coatings as potential sources of microplastics, there are not enough studies reporting the status of microplastic pollution derived from ship paints. Qualitative and quantitative evaluations are possible only after the chemical and material property data of ship paint-derived microplastics are well accumulated, such as polymer types and elemental compositions. To establish a spectral library of ship paint-derived microplastics, a total of nine types of representative ship paints (two types of primer as anticorrosive paint, two types of tie-coat as adhering agent between the primer and top-coat, and five types of top-coat as antifouling paint and deck paint) were selected according to their functions and working mechanisms. Each dried paint was pulverized into secondary microplastics using a mortar under liquid nitrogen and sieved through a 2 mm-sized mesh to simulate environmental samples. The FTIR spectrum of the secondary microplastics was measured and the spectral information on the absorption peaks, their appearance, corresponding functional groups with mode of vibrations, as well as the fingerprint of specific constituents, was reported in this presentation. When blind samples were analyzed after library construction, they showed an average matching rate of 96.0% or more with the established library spectrum. In addition, the external characteristics and constituent element information of secondary microplastics were analyzed by using SEM and EDS. As a result of observing the external image through SEM, it was found that copper chunks were embedded in some antifouling top-coats. In addition to C and O, which are the most dominant elements in all types of ship paint-derived microplastics, Mg and Si were clearly detected in primers; Si, Ti, and Ba in tie-coat; Cu, Zn, Fe, and Ca in antifouling top-coat; Mg, Si, Ca, Fe, and Ba in epoxy based top-coat; and Ca and Ti in urethane based top-coat. Their relative compositions were unique among different types of paint-derived microplastics.

Establishing a library on the polymer type by FTIR, external features by SEM, and elemental compositions by EDS will contribute to clearly identifying ship paint-derived microplastics in environmental samples, and thus to better quantitative analysis required for risk assessment.

### 3.25.P-We274 How Precisely can we Analyse Very Small Microplastic Particles?

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When considering their presence in environmental samples, the number of microplastic particles (MP, polymer fragments from 1 µm to 1 mm diameter) [10.1021/acs.est.8b05297] is expected to increase exponentially towards smaller sizes. The precision of an analytical method that aims for fast results in this size range is therefore of great concern. *TUM-ParticleTyper* [10.1371/journal.pone.0234766], an open-source software that detects objects as small as 10 µm in dark-field images of particles deposited on a filter, has now been further developed and extended by the Random Window Subsampling (RWS) approach [10.1007/s00216-021-03326-3] to enable the analysis across the entire MP size range, whereby particle identification is performed automatically by Raman microspectroscopy (RM). Here, we report on the precision of this procedure estimated for the particle size range 1 µm—50 µm.

Suspended microplastic particles were generated [10.3389/fchem.2020.00169], including three polymer types (PS, PET, PLA). Particles of a sample were separated out using a Au-coated polycarbonate filter (APC GmbH, pore size 0.8 µm). On the filter loaded this way, five repetitive measurements were conducted by applying a fully automated procedure supported by the in house developed software *TUM-ParticleTyper 2* (average duration 21 h). Particle detection and Raman measurements (532 nm, 3.5 mW, 40 × 0.5 s per particle, 100 × magnification, N. A. 0.9) were hereby started iteratively, until the given minimum precision requirements were met. The results, obtained under consistent conditions, give  $(5.35 \pm 1.29) \cdot 10^5$  plastic particles ( $2.132 \times \text{SD}$ ). Each identified between 660 and 840 plastic particles by covering about 0.15 % of the total filter surface, followed by extrapolation. Here, a relative error of 24 % is achieved within 21 h of measurement time per result. Uncertainty can also be estimated on the basis of a single result as calculated from bootstrap samples of RWS, considering only the quantification of particles made of certain materials (plastic types in this case). The uncertainty of each extrapolated result was between  $0.43 \cdot 10^5$  and  $0.57 \cdot 10^5$  plastic particles (CL 90%). As the selection is not limited to plastic types, the procedure is also generally applicable for universal particle analysis beyond MP. These results provide a first indication of the random variation to be expected as the general methodology of microplastic analysis is extended to smaller particle sizes (> 1 µm).

### 3.25.P-We275 Investigation of Airborne Microplastics and Heavy Metals Interaction in Coastal Environment: Application of SEM + SEM EDS, Raman, and LIBS-MS Instrumentation

**Precious Onyinyechi Odika**, School of Environment, Geography and Geosciences, University of Portsmouth, United Kingdom

Microplastics (MPs) are classified as plastic particles with a size range  $\leq 5\text{mm}$  in diameter. Pollution by MPs is gaining more attention given its potential cause of toxic accumulation in the food chain, which can pose risk to humans and animals. Of concern also is their ability to adsorb and transport heavy metals (HMs). Heavy metal adsorption by MPs can be affected by the formation of biofilms, and the ageing processes and types of MPs. Although some studies have identified MPs as potential vectors of heavy metals, the adsorption mechanism in the complex natural environment where many heavy metals coexist is yet to be fully investigated. Understanding the chemical interactions between airborne MPs and HMs will improve the present knowledge on the ecological effects and on the toxicity of MPs at environmentally realistic concentrations, their weathering pattern and risk

implications. This ongoing PhD study will develop some much needed instrumentation and methodological techniques and standards for airborne MPs research; sampling, analysis, assessment, interpretation, monitoring and management. It will involve a combination of field and laboratory activities, and the use of Deployable Particulate Sampler (DPS), SEM + SEM EDS, Micro-Raman and LIBS-MS analytical techniques. We will investigate the chemical interactions of different polymer types of MPs with toxic chemicals under various climatic and weathering conditions and their potential resistance level. Specifically, there will be a regional and seasonal comparative analysis of heavy metals transport and adsorption by MPs in the coastal areas of Portsmouth in the UK and Lagos in Nigeria, West Africa. There will be a comparative evaluation of experimental approach versus active method. In addition, simulations and modelling of exposure assessment will be done to predict possible human health and environmental risk from potential airborne MPs pollutants. The regional comparative study will help to evaluate climatic and or geographical sensitivity in the characterisation of MPs with respect to toxic chemical adsorption, desorption and transport. It will contribute to designing global airborne MPs instrumentation development framework and quantitative measurements for global air quality monitoring and management.

### **3.25.P-We276 Identification of Micro- and Nano-plastics Using Raman Spectroscopy Coupled with Confocal Microscopy**

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Plastic pollution is ubiquitous and persistent in the aquatic environment where a mixture of plastic particles is found.

Environmental plastic particles are heterogeneous in terms of both physical and chemical properties. Nanoplastics (dimensions < 1 µm; NP) are expected to be widely present in the environment, but there is a paucity of reliable data on the concentration and distribution of small microplastics (dimensions < 10 µm; sMP) and NP in environmental matrices notably because of sampling and analytical challenges. Accordingly, there are knowledge gaps in understanding their effects and interactions with biota under environmentally relevant exposure conditions. The current lack of relevant and standardized methods for quantification of sMP and NP precludes the development of robust environmental risk assessment strategies.

Chemical identification and quantification of plastic particles is possible using vibrational spectroscopy such as Raman spectroscopy coupled with confocal microscopy. It enables chemical characterization and counting of particles with dimensions between 1 and 10 µm and also eventually particles with dimensions in the sub-micron range. At first, laboratory-based samples using two different stages (pristine and aged) of polyethylene were used. To enhance environmental relevance, comprise milled plastic particles with a variety of morphologies and broad size distribution were used. Then, the complexity of the samples will be increased to test the method on a mixture of milled particles with sizes smaller than 10 µm. The strategy will be further tested and optimised for environmental samples after relevant sample preparation.

This work focuses on the detection and characterization of small microplastics and nanoplastics using Raman microspectroscopy to be able to determine the concentration and distribution of these particles in environmental matrices. The results will facilitate determination of realistic concentration input values for further ecotoxicological studies regarding plastic particles with size smaller than 10 µm. Our work will thus yield environmentally relevant sMP and NP exposure data thereby enabling consistent risk assessment.

### **3.25.P-We277 Combining Polymer-specific Binding Peptides and Asymmetric Flow-Field Fractionation Analytics to Tag and Detect Nanoplastics in Mussel's Haemolymph Circulating Cells**

**Marisa Sarria Pereira de Passos<sup>1</sup>, Cloe Desmet<sup>2</sup>, Dora Mehn<sup>2</sup>, Vanessa Modesto<sup>3</sup>, Nicoletta Riccardi<sup>4</sup>, Maximilian Nöth<sup>5</sup>, Tetiana Kurkina<sup>5</sup>, Francisca Contreras Leiva<sup>5</sup>, Ulrich Schwaneberg<sup>5</sup> and Douglas Gilliland<sup>2</sup>**, (1)EU Commission JRC, Italy, (2)European Commission Joint Research Centre, Italy, (3)IRSA CNR Verbania: Istituto di Ricerca sulle Acque, Consiglio Nazionale delle Ricerche, Italy, (4)Water Research Institute - Italian National Research Council IRSA-CNR, Italy, (5)RWTH Aachen University, Germany

Bivalve mollusc's haemolymph is one ecologically relevant biomatrix that can be extracted non-invasively to assess the presence of nanoplastics. It constitutes the first line of defence against the entry of external alien material and therefore pertinent to indicate the health status of specimens and populations in natural ecosystems. Haemocytes play a key role on the innate immunity, transporting nutrients, respiratory gases, enzymes, metabolic wastes and toxicants throughout tissues. Induction of functional or stress responses and apoptotic processes on these circulating cells occur particularly fast, and thus makes them worthy of study to evaluate their suitability for aquatic biomonitoring programs.

Tagging and detection of unmodified nanoplastics is technically challenging due to the lack of analytical methods, which can reliably distinguish polymeric particulates from other non-polymeric particulates. An often-cited solution is to dope or stain the nanoplastics with optical markers prior to analysis, but this could be a source of artefacts if marker molecules leach from plastic particles or if interactions are not sufficiently specific towards the targeted polymeric materials. Furthermore, this labelling strategy adds an additional step since, prior to their use in testing, any unbound marker molecules have to be efficiently separated from the polymer without further disrupting their binding to the nanoplastics.

Flow Field Fractionation techniques allow efficient separation of liquid dispersed particulates from free molecular species while also permitting size-fractionation across the size-range from a few nanometers to micrometers. Since these methods can use simple aqueous dispersions as the mobile phase, including those mimicking the physiological conditions, size separation by Flow Field Fractionation techniques is therefore compatible with any biomolecular species as peptides and proteins, which may be associated with the polymer particles.



In this work, Asymmetric Flow-field Fractionation coupled to different on-line detectors (UV-vis, fluorescent and dynamic light scattering) was investigated as a mean to size-separate and detect nanoplastics in complex biomatrices (mussels haemolymph), which have been incubated and tagged with polymer-specific biomolecular species fused with a fluorescent protein. Results evaluating the binding specificity of different polymer-specific binding peptides are presented and discussed.

### 3.25.P-We278 Fluorescent Micro- and Nanoplastics for the Optimization of Analytical Pathways in Various Biological Systems

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Within the past decade, research focused on attempting to understand the interaction of micro- (1  $\mu\text{m}$  – 5 mm), submicron- (100 nm – 1  $\mu\text{m}$ ), and nanoplastics (< 100 nm) with humans or various animals has been of increasing interest. These studies seek to determine if any interactions which may occur will have adverse impacts. However, with this growing interest has come a growing need to optimize sample preparation and analytical protocols that facilitate detection of plastic particles in such complex biological systems. In an effort to address this need, our study focuses on the creation of micro- and nanoplastic particles with and without a fluorescent label to use as a foundation for optimizing sample analysis. Microplastic particles of poly(ethylene terephthalate) and polypropylene with sizes < 300  $\mu\text{m}$  were prepared and used to develop a protocol that facilitated the use of correlative light, electron microscopy with Raman Spectroscopy to detect the microplastics after their ingestion by medusa of the jellyfish *Cassiopea andromeda*. Finally, the microplastic particles could be used as a stock material to create nanoplastic particles with average sizes near 75 nm. As a proof-of-concept, these nanoplastics were utilized in *in-vitro* experiments aimed at mimicking potential interactions with cell types relevant to the human gastrointestinal system. Uptake by two different cell types; human colon (colorectal adenocarcinoma) cell line Caco-2 and mouse monocyte/macrophage cell line J774A.1; could be observed.

### 3.25.P-We279 Production and Analysis Methods for Pristine and Degraded Microplastic and Nanoplastic Reference Materials

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Most microplastic (MP) and nanoplastic (NP) environmental fate and hazard assessments utilise spherical, monodisperse polymer particles that do not represent the continuum of partially degraded, irregular-shaped MPs in the natural environment. As such, there is a strong need for environmentally relevant test and reference materials (TRMs). Cryomilling approaches can readily generate bulk amounts of MP TRMs >100  $\mu\text{m}$ , but only negligible amounts of TRMs below this size and into the nanoscale (generally <<0.1% by mass). New methods need to be developed for the production of small MP (sMP; 1-100  $\mu\text{m}$ ) and NP (<1  $\mu\text{m}$ ). In the current study, pristine polypropylene (PP), polyethylene (PE, HDPE and LDPE), polyethylene terephthalate (PET), polystyrene (PS) and Polytetrafluoroethylene (PTFE) pellets were cryomilled and sieved to produce core stocks of TRMs <100  $\mu\text{m}$  in size. Characterisation by a Morphology G3 particle size and shape image analyser showed that all TRMs had a mean particle size of ~100  $\mu\text{m}$  by volume and 0.5-2  $\mu\text{m}$  by particle number, indicating high numbers of sMP and NP, but representing a very low mass. Cryomilled PET, PE and PS were selected for use in the development of a secondary TRM production step that combined UVC ozonation to partially UV degrade the surface of the particles, followed by probe sonication to promote further fragmentation. At the same time, a hyphenated field flow fractionation (FFF) and pyrolysis GC-MS workflow was developed for the mass-based quantification of sMP and NPs of defined size fractions. This involved the use of solvent extraction of the particles from aqueous dispersions prior to analysis and quantification. The approach was able to extract ~100% of sMP and ~80% of NP from aqueous dispersion and quantification limits were identified as ~0.1 ng, ~1 ng and ~1  $\mu\text{g}$  for PET, PE and PS respectively. Morphology G3 was used to quantify the number of sMP, while nanotracking analysis was used to quantify the size distribution and number of NPs. Results suggested that the UVC ozonation and probe sonication strongly increased the quantity of sMP and NP, with mass fraction increases of 30x (PS), 15x (PET) and 6x (PE), respectively. However, this still represented  $\mu\text{g}$  quantities being produced from mg quantities of stock material. The results suggest that environmentally relevant sMP and NP TRMs can be produced, but the method requires optimisation to generate quantities suitable for use in environmental fate and effects studies.

### 3.25.P-We280 Key Challenges and Limitations to Characterizing and Quantifying Environmental and Human Health Risks to Micro- and Nanoplastic Particles: Reference Materials Urgently Required

**Todd Gouin**, TG Environmental Research, United Kingdom

Micro- and nanoplastic particle (MNP) research is at a nascent stage, with numerous studies observing a need to adopt robust quality assurance / quality control (QA/QC) practices regarding sample collection, analysis and effects testing. Good QA/QC is needed to support the reliability and relevance of data generated, which further supports comparability across studies and which further strengthens the ability to perform a risk assessment. It is generally understood that an important element of QA/QC protocol relates to a demonstrated understanding of the characteristics of the stressor under investigation. The development and application of sampling and analytical methods, for instance, relies on the use of analytical standards, which are used to quantify the efficacy of the sampling and analytical method, such as in the reporting of recovery efficiencies or in the use of quantifying calibration curves. At present, there are no readily available standardized MNP reference materials or methods. In an effort to address this urgent research need, a multi-stakeholder workshop was held during May 2022 in Atlanta, aimed at exploring opportunities to support the generation of a suite of environmentally relevant standard reference MNP materials for use to support

the validation of sampling, preparation, and analytical protocols. MNP reference materials would encompass different resins, morphologies, and sizes to represent in some degree the particle variability present in the environment. Standard materials would serve a variety of needs, but would be particularly valuable in supporting the adoption of good QA/QC practices for both environmental monitoring and effects testing, thus helping to strengthen the quality and reliability of data to support risk-based decisions. This presentation will summarize key output from the workshop, including a summary of the various approaches currently used to generate and weather MNP, their strengths and weaknesses and recommendations regarding best practices for use of MNP reference materials for supporting both analytical method development and effects testing.

### **3.25.P-We281 Untangling Environmental Ageing Processes of (Micro)Plastic Toward the Creation of Realistic Reference Materials**

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The creation of reference materials mimicking the properties of environmental plastics is needed to improve assessment of environmental risk and harmonization of analytical methods. The study of ageing processes is therefore gaining interest of researchers, but the effects of different processes determining ageing (i.e., physical, chemical and biological factors) are still only understood to a limited degree. Here we propose the test of multiple ageing stressors on (micro)plastic specimens to observe how different processes affect (micro)plastic properties. Three different plastic objects composed by polyethylene (PE), polypropylene (PP) and polylactic acid (PLA) were tested. These materials were aged using 3 factors: ultraviolet (UV) irradiation to simulate photoinduced oxidation, the incubation with a mixed algae inoculum to simulate biofouling and both treatments performed sequentially. Changes in surface characteristics were assessed by Fourier-transform infrared spectroscopy, scanning electron microscopy, and water contact angle. Moreover, biofilm growth on plastic fragments was analyzed by chlorophyll fluorescence and optical microscopy. UV radiation caused a significant increase in the density of oxidized groups (i.e., hydroxyl and carbonyl) and increased the surface wettability of PP and PE, while only slightly affecting the surface properties of PLA. Biofouling too, strongly affected surface alterations, regardless of the polymer type and previous ageing process. All biofouled samples present (i) specific infrared bands of new surface functional groups (e.g., amides and polysaccharides), (ii) the diffuse presence of algal biofilm on the plastic surface, and (iii) a significant decrease in surface hydrophobicity. The monitoring of biofilm growth showed that the surface properties of plastic affect cellular attachment and growth rates, whereby hydrophobic samples (i.e., pristine PP and PE) present faster colonization by algae and more abundant biofilm on their fragments. This suggests that chemical and biological alterations are strongly affecting plastic features and that these processes should be considered to foster realistic and reliable reference materials.

### **3.25.P-We282 Production and Characterisation of Environmentally Relevant Microplastic Reference Materials for Agricultural Soils**

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Soil environments, particularly in agricultural settings, are often contaminated with microplastics. Agricultural plastics, such as mulching films, are used in close or direct contact with soils and there is growing evidence demonstrating that they represent a potential source of microplastics. These particles, especially those derived from films composed of conventional non-degradable polymer materials, may accumulate in soils, leading to increasingly high concentrations over time. There is a demand to undertake fate and effects studies to understand the behaviour and impacts of this contamination. Yet, there is a lack of reference materials available for this purpose.

Microplastic reference materials can be defined as standardised and well-characterised batches of particles available for use in scientific testing. Their scarcity in this context is related to several challenges in producing environmentally relevant particles, which has been highlighted as an important requirement for microplastic reference materials. This includes challenges in generating microplastic particles from relevant source materials and obtaining environmentally relevant particle typologies (e.g. size, shape, surface properties). There is also lack of consensus or guidelines regarding the necessary particle and material characterisation. This poster presents an overview of batches of microplastic reference materials produced from environmentally relevant source materials, including results of a thorough characterisation of the microplastic particles generated. Advances in micronising agricultural mulching films are described. This includes an emphasis on the efforts made to generate sufficiently large batches of reference materials that can be used across multiple experiments and by several research groups, therefore facilitating harmonisation of fate and effects testing. This work represents an ongoing activity to develop new typologies of microplastic particles from different source materials to supply environmentally relevant microplastics for scientific experimentation.

### **3.25.P-We283 Generation and Characterization of Cryomilled Micro- and Nanoplastics**

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There is a critical need to generate environmentally relevant Microplastics (MPs) and nanoplastics (NPs) to better investigate MP behavior in laboratory settings. Environmental MPs are heterogenous in size and shape unlike monodisperse and uniform microspheres commonly used. Cryogenic grinding, or cryomilling, was successfully utilized to transform polystyrene (PS) bulk material into heterogenous micro and nano fragments. Fourier-Transform Infrared (FTIR) spectroscopy confirmed that this

approach did not alter polymer surface chemistry. The number of milling cycles (time of milling) and frequency of grinding (intensity of milling) were varied to investigate the role cryomilling parameters had on generated MP characteristics. The resulting particle size distributions and surface area of these varied cryomilled samples were measured and compared. Coulter Counter and Nanoparticle Tracking Analysis (NTA) were employed to measure the particle size distributions at the micro and nanoparticle size ranges, respectively. Microspheres were used to determine what camera settings yielded more accurate sizing and to reduce bias in the NTA analysis. Increasing milling cycles generally increased the number of smaller particles. As PS was cryomilled, small nanosized fragments broke off from larger MPs. As the number of NPs increase over time, the diameter of larger MPs decreases. The number of milling cycles was observed to more consistently impact the size distributions of fragments compared to the frequency of milling. This study offers both cryomilling analysis and recommendations to generate and make use of these MP/NPs in more environmentally relevant work.

### 3.25.P-We284 Synthesis and Characterisation of Polystyrene Particles with Silver Nanoparticle Core for Environmental Fate Studies

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Recently, research efforts have increased to understand the fate and impact of plastics in the soil environment. Due to the background of natural organic matter in the environment, the quantification of polymers in environmental matrices is challenging. Therefore, the use of well-characterised model particles is mandatory to assess the fate of nanoplastics in soil. The quantification and localisation of these particles are alleviated by doping with metals with very low natural background in the environment, e.g. palladium (Pd) or silver (Ag). In this work, we follow Mitrano et al. and synthesise polystyrene particles (PS-P) in the range of up to 300 nm doped with Ag as tracer material. This synthesis uses the well-characterised and commercially available reference silver nanomaterial NM-300K as precursor. The silver nanomaterial was coated with a thick shell of polystyrene following the work of Braconnot et al. to prevent Ag release from the particles and ensure that the particle surface properties match polystyrene, which is mandatory to investigate the PS-P interactions in environmental matrices. We use only the essential educts NM-300K, styrene, divinylbenzene and K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> to exclude effects from comonomers, other additives, or contamination from reducing agents in environmental fate studies. The synthesised particles were characterised by light scattering methods and electron microscopy. The particles' Ag content and the potential Ag release were analysed by inductively coupled plasma optical emission and mass spectrometry after aqua regia digestion. The silver nanomaterial with the nominal diameter of 15 nm was successfully coated with a symmetric polystyrene shell with a thickness of approx. 130 nm. Stability experiments show excellent colloidal stability with a zeta potential of approx. -40 mV and a stable hydrodynamic diameter of approx. 260 nm. Under light acidic conditions, the chemical stability of the particles was satisfactory with a very low Ag release. This implied the good applicability of the synthesised particles for environmental fate studies.

### 3.25.P-We285 Artificially Aged Nanoplastics Models for Laboratory Testing

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Reference particle models are essential to obtain an accurate perspective of how environmental nanoplastics behave in natural systems and to generate data on their environmental fate and impact on living organisms. However, although the current available models are particularly important for filling the initial knowledge gaps on nanoplastics, they do not demonstrate enough diversity and/or accuracy to represent the actual heterogeneity of the physical and chemical properties of environmental nanoplastics.

In this framework, the Joint Research Centre (JRC) and the National Institute of Standards and Technology (NIST) are collaborating on a challenging project, which aims to identify a novel strategy for the production of aged nanoplastics. These new test materials are specifically designed to become environmentally relevant models. To reproduce in a controllable fashion both morphological and surface chemistry characteristics of nanoparticles distributions different top-down synthesis approaches are investigated. The model particulate is synthesized (i) via mechanical abrasion (cryomilling) of artificially aged plastic specimens (heat and UV exposure) and (ii) via laser ablation of plastic targets in chemically reactive liquid environment. The pros and cons of two approaches are compared and discussed from the synthesis and the surface chemistry point of view. The objective of this investigation is to obtain nanoplastics, with a reasonable throughput, which mimic naturally weathered ones in size distribution, morphology and surface chemistry.

The scalable production of these nanoplastics is investigated to make them available to stakeholders. This test material will have applications for the development of new analytical methodology to detect nanoplastics, to better understand their role in carrying other pollutants and increase knowledge related to their behaviour and fate in the environment.

### 3.25.P-We286 Fabrication of Controlled Microplastic Materials for Pulmonary Toxicity Studies

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Microplastic (MP) particles and fibres can be produced from the breakdown of larger plastics and their presence in ambient air requires research into potential toxicity following inhalation. The majority of recent toxicological studies primarily use commercially available polystyrene beads which are not representative of the range of microplastics which occur in the environment. To address this, a methodology has been developed to create MP particles and fibres of a respirable size (<5 µm)

and irregular shape for use in pulmonary toxicity studies which will help answer questions about how physicochemical properties influence toxicity. Polyethylene terephthalate (PET) (Sigma), polyamide 6,6 (PA6,6) (Sigma), and polystyrene (PS) (GoodFellow) were chosen as the test polymers. PET, PA6,6, and PS were dissolved in hexafluoro-2-isopropanol, formic acid, and tetrahydrofuran respectively. Precipitation was performed through the addition of ethanol under probe ultrasonication. Particles were passed through a 5 µm sieve and centrifuged to remove over- and undersized particles. Fibres were fabricated through electrospinning of the same plastic solutions, a process using an electrical gradient to extrude polymer fibres with a diameter of 1-2 µm. Cryotome slicing was then performed to yield fibres with an aspect ratio >3. Both MP types were washed several times to remove residual solvent and other potential contaminants and left to dry. Size distribution was performed through a combination of SEM (Zeiss LEO 1525), and Dynamic Light Scattering (Zetasizer Pro), and fabricated particles were found to all have an aerodynamic diameter of <5 µm. Pyrolysis- Two-Dimensional Gas Chromatography Mass Spectrometry (MS), inductively coupled plasma MS, and Limulus Amebocyte Lysate assays were performed to test for methodological artefacts that may interfere with *in vitro* studies. This simple method for the fabrication of pristine MPs provides a resource to ask fundamental questions about how shape, size, and polymer chemistry influence pulmonary MP toxicity. These techniques can be adopted by the wider MP community to investigate more MP physicochemical properties than those commercially available. Preliminary *in vitro* toxicity studies using fabricated PS particles and fibres caused no significant decrease in cell viability after 24 hours of exposure at doses 3.125-100 µg/mL. This will be supplemented by a comprehensive screen of all fabricated MPs in a macrophage-like cell line.

### 3.25.P-We287 Controlling Contamination: A Comparison of Control Data Analysis Methods

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After more than a decade of research, the harmonisation of methods has become an international priority in the field of environmental monitoring for microplastics (MPs). This harmonisation has focused predominantly on extraction and isolation methods, identification of MPs and, more recently reporting. However, there is currently no consensus on how the data generated from controls and blanks are used to correct the sample data. Common approaches used in microplastics studies include a) No correction; b) Subtraction; c) Subtraction of mean; d) Subtraction of LOD/LOQ; e) Spectra similarity; or f) Statistical analysis. This study evaluated 51 different data correction strategies based on variations of the 6 approaches listed above to determine the most suitable for application to MP datasets. These methods were tested on a dummy dataset which comprised of real background contamination collected from *in situ* laboratory analysis. Of the 51 data methods tested, only 7 managed to remove on average 95% of the contaminant data. Data correction methods based on total subtraction were unable to remove even 50% of the dataset, and hence are not recommended for use to correct MP datasets. All methods based on average subtraction, LOD/LOQ and spectra similarity were successful at removing at least 50% of the data. We recommend using the LOD/LOQ method combined with spectra similarity to remove known contaminants within MP datasets. However, the spectra similarity method relies on all items being analysed spectrally. Therefore, if all items are not spectrally characterised, the LOD/LOQ method without a spectra similarity step was also found to be acceptable.

### 3.25.P-We288 Interlaboratory Study on the Analysis of Microplastics

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Within the European quality Controlled Harmonization Assuring Reproducible Monitoring and assessment of plastic pollution (EUROqCHARM) project an interlaboratory comparison study (ILS) has been organised for the analyses of MP in environmental matrices. The aims of the study were to assess the variance between laboratories performing MP analyses, to assess the different methods used for MP analyses, and to assess the performance on the different type of methods used. With the overall aim of the study to move forward to validate and harmonize various methods.

The ILS focused on the analyses of MP in environmental realistic contaminated test materials. The samples included 'soda' tablets simulating water samples with the different polymers in the size region of 50-299 µm, and three sediment and sand samples.

98 laboratories subscribed to the study of which 67 submitted data. Most those laboratories reported on the number of particles (81-87%, depending on the matrix), and only 17-21% reported on the mass of MPs (i.e., mg/tablet or mg/kg). A diversity of methods was used for the extraction and analyses of MP.

Since only a small number of participants report on mass, most of the results on mass were insufficient to be able to perform any statistics on. For the reporting on number of particles, the variation (expressed as relative standard deviation (RSD)) in results was 51-130%, showing that the performance on MP analyses did not improve compared to previous ILS even though the identity of the polymers was known on forehand.

This high variation shows that there is a need for harmonization of methods for MP analyses, and a need for training in order to achieve a better agreement between laboratories performing MP analyses in environmental matrices.

### 3.25.P-We289 Value for Money: A Cost-effectiveness Analysis of Microplastic Sampling and Analytics

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The quest for increasingly small microplastic particles, together with their potential impact on ecosystems has expedited the

development of microplastic research in recent years. A wide range of sampling procedures, sample processing steps and sample analysis techniques, both manual and automated, have been established. Despite this progress, this diversification of techniques impedes cross-study comparability and can be confusing for researchers. Many of the currently applied procedures are also perceived as expensive. Unanswered questions concerning MP abundance, composition, distribution and fate in the marine environment emphasize the need for standardised and reliable monitoring procedures to comply with the Marine Strategy Framework Directive (MSFD).

In our study, which was performed within the JPI Oceans Andromeda project, we performed a cost-effectiveness analysis (CEA) of frequently used methods for microplastic analysis in seawater on a European scale. Data was collected through an online survey consisting of 97 questions related to sample acquisition, sample processing, and sample analysis of preset scenarios. In these scenarios, seawater samples were defined with specific information on microplastic load and composition, microplastic size range, and suspended particulate matter concentration. Total working hours, personnel costs, sector of employment, European marine region of employment, and equipment costs/depreciation/usage were also included in the survey. The survey was performed during autumn 2022 and was spread to experts in the field through personal contacts in various European microplastics expert groups. Following the survey, key outcomes were discussed during two different workshops held with microplastics researchers and with policy makers. Their opinions and perspectives were then used to write up recommendations. The survey allowed us to compare relative costs and outcomes of different methods based on real experiences of experts in the field. Obtained results allow to gain insight on which workflows provide the greatest value for money for seawater samples, as well as on key elements to which the CEA outcome is sensitive. By providing concrete and useful recommendations of monitoring strategies in terms of cost-effectivity, we want to support researchers, policy makers and other stakeholders when choosing between different microplastic workflows during future monitoring campaigns in the scope of the MSFD.

### **3.25.P-We290 Microplastic Analytical Proficiency Testing: Using Immobilised Particles to Improve Experimental Designs**

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Interlaboratory calibrations (ILCs), or ring trials, are important procedures to compare and validate the analytical proficiencies of different microplastic (MP) laboratories, techniques or instruments. Conducting an ILC, conventionally involves the preparation of artificial test samples. Every participant will obtain and measure an individual sample and report the result for comparison. There is a variety of published experimental designs for the preparation of the test sample either repetitively, or by splitting a common stock sample. However, especially when the tested material involves particles in the lower micrometer size ranges (here termed small microplastics, sMP, e.g. ~1 to 100 µm), an independent validation of the MP quantities in each replicate is not possible: the only techniques available to quantify them would be those that are supposed to be evaluated by the ILC itself. We introduce the idea of immobilised MP samples and demonstrate their implementation for ILCs. The conceptual difference to suspended ILC designs is that the same sample, in the same constitution, gets measured by every participant instead of individual replica.

The presented implementation is based on cryo-milled and sieved irregular MP particles (nominal sizes of 10 – 70 µm), which are filtered and arrested on silicon and aluminium oxide filters: two kinds microspectroscopic filter materials frequently used for MP quantification. Prior to filtration the particle suspension is mixed with a liquid inorganic binder which leads to an immobilisation of the particles after curing that can withstand flushing with running water or compressed air.

The intended effect of the switch from a design of repeated replica to a single immobilised sample is to exclude error terms from an ILC which are a) non-controllable, and b) specific to the ILC itself, thus not informative about the proficiencies in measurements of real world samples.

Preliminary results suggest that in a comparison between a conventional state-of-the-art suspended ILC design and one using an immobilisation, the relative standard deviation of the results reported by the participants was higher in the former by a factor of 6 to 7.

We also discuss other fields of application for the concept of immobilised MP particles: it has already been utilised for sMP purification method evaluations, but also MP sample storability, and correlative microscopy could benefit from the idea.

### **3.25.P-We291 Platinum Vaporization-deposition Coated Polycarbonate Membranes For Comprehensive, Multimodal, and Correlative Microscopic Analysis of Micro-and Nanoplastics and Other Environmental Particles**

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Anthropogenic particles, including microplastics, are sampled from many matrixes, including the environment such as water, sediment, and air. However, there are no standard methods for sampling particles in the environment; thereby, many different approaches are used for both single particle and ensemble distribution or bulk chemical analyses.

For microplastics, the most commonly used sampling techniques are bulk sampling, i.e., the entire sample volume is collected and not reduced during the sampling, or volume-reduced sampling, i.e., the sample is reduced while collected, such as through filtration. In both cases, the particles of interest will end up on filters. The filters are then analyzed visually and spectroscopically. For particles larger than 300 nm, the quality of the filters is not as crucial as for smaller-sized particles. The size distribution of particles collected in the environment shows a higher abundance of particles within the smaller-sized classes. Because of their small size, high abundance of particles, and a potential biased impact from the operator, automated analysis are required. For automated analysis, the filters' surface has to be unstructured and smooth and have good optical contrast, low Raman, and

fluorescence signal, especially in the range of polymer bands. If Scanning Electron Microscopy (SEM) analysis is desired, the conductivity of the filter and particles is important, especially under high vacuum conditions. The filters should also not be affected by high pressure from the filtration, treatments to reduce organic matter, and have a defined pore size and pore distributions and not be fragile. Preferable many filters made of the same material but with different pore sizes can be used during the sampling of particles. Subsequently, the filters would be put through the analysis pipeline with one or another mode of microscopy or even correlative microscopy workflows.

We aimed to develop filters made of the same material and have a defined and practical pore distribution, available in many pore sizes and with a circular diameter of 47 mm and 25 mm. Moreover, the membranes must be able to handle high pressure, filtrate a large volume of solutions, withstand chemical or enzymatic treatments, low background signal from filters that do not interfere with the polymeric fingerprint as well as good properties in SEM with various detectors and performing good in different imaging modes of light microscopy (LM).

### **3.25.P-We292 Characterization of Microplastics in Drinking Water Using Micro-ftir: Method Validation**

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The term 'microplastics' (MP) commonly refers to solid and insoluble plastic particles of small dimensions, usually smaller than 5 mm (and without a specific lower limit). It is also common to distinguish between primary and secondary microplastics.

Regardless of their classification, MP contamination is an environmental issue of increasing concern.

Water suppliers using surface water as a raw water resource are likely to be affected by the potential presence of MP particles. As investigated in many studies, larger particles are retained during depth filtration, bank filtration, artificial recharge, underground passage, and membrane filtration. Data on the occurrence of very small microplastic particles in freshwater systems and their behavior during water treatment still need to be included at this stage. There must be comprehensive studies on microplastics in raw water resources, their behavior during drinking water treatment, and their potential occurrence in drinking water.

The need for harmonized methods, reference material, and thus comparative data is one of the biggest challenges regarding determining MP in the environment. Therefore, efforts must be taken to evade these challenges, mainly in drinking water.

Destructive techniques such as pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS) have been employed in the chemical identification of microplastics, but no information on shape or size of particles can be collected. Non-destructive techniques such as optical microscopy, scanning electron microscopy, SEM, and fluorescence microscopy have been employed for the quantification of microplastics.

Nevertheless, these techniques cannot allow the identification of the polymer composition of these particles. Micro-FTIR (Fourier Transform Infrared Spectroscopy) is a well-known technique that combines infrared spectroscopy with an optical microscopy approach; it is a non-destructive and reproducible technique.

The novelty of our study is to develop and optimize an analytical method for the quantification and the simultaneous identification via Micro-FTIR of different MP, which is present in drinking water.

Regarding the optimization and validation method, several parameters were evaluated, namely: i) sampling method; ii) sample size; iii) sample processing and storage; iv) laboratory preparation; v) clean air conditions; vi) negative controls; vii) positive controls; viii) sample treatment; ix) polymer identification.

### **3.25.P-We293 Taking Plastic Particle Extraction to the Next Level: Development of an Extraction Process for Nanoplastics from Compost and Sample Preparation for AFM Analysis**

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Microplastics are ubiquitous in the environment and several extraction processes for micrometer-sized particles from complex environmental matrices have been reported during the last years. Raman/IR and fluorescence microscopy techniques with fluorescent labelling are the most common techniques for counting extracted particles but have limited size detection ranges. So far, little is known about the fragmentation and degradation of microplastics into secondary nanoplastics under environmental conditions, mostly due to the lack of reliable nanoplastic extraction protocols for complex environmental matrices and analytical methods needed for quantification and identification.

We present preliminary results on an extraction process developed for nanoplastics from compost, which is based on deagglomeration and a sieving cascade to isolate coarser fractions from the nanoplastics, which are then enriched by a density-driven process adapted from the harvesting of mitochondria in cell biology. To test the extraction efficiency during method development we used spherical fluorescent polystyrene (PS) beads (0.91 µm). This model polymer particle was also beneficial for the AFM study. We could easily differentiate the polymer and compost particles by morphology and adjust the filtration and application parameter. The premise for accurate differentiation of nanoplastics from remaining compost and quantification is a homogeneous particle sub-monolayer on the substrate used for AFM analysis. In the stepwise approach to real-world samples, we then applied the optimized extraction process also on pristine and UV-aged polyamide (PA) nanoplastics, which are polydisperse, non-spherical, and surface-aged. The AFM material contrast, mapping of mechanical properties or adhesion can be used for the differentiation purpose. The developed extraction process, the controls and substrates finally shall enable AFM-nanoIR, to differentiate particles of natural origin from those with synthetic polymer composition, but also SEM cathode luminescence is an option to resolve both chemical composition and particle size.

### **3.25.P-We294 Enabling Technology for Routine Microplastics Sampling and Analysis: Autonomous Marine Drones and High-Throughput Hyperspectral Imaging**

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Monitoring the occurrence of microplastics (MP) in the environment is critical to 1) understand exposure for risk analysis of ecosystems, 2) elucidate transport processes, and 3) to monitor the effectiveness of pollution reduction measures. Current MP sampling and analysis techniques do not allow high sample throughput due to bottlenecks in both sample collection and sample analysis. We conducted a proof-of-concept study on the west coast of Norway to assess the viability of combining rapid marine drone-enabled sampling with high throughput short-wave-infrared hyperspectral imaging (HSI) analysis of MP. We propose a workflow for surface water sampling of MP which uses an uncrewed surface vehicle (USV) to collect triplicate manta-net samples simultaneously and in a repeatable fashion. Using HSI, we demonstrate the capacity to analyze multiple MP samples (down to 500  $\mu\text{m}$  particle diameter) in a short time frame (20 x 47 mm  $\varnothing$  filters/h). A multivariate model rapidly classifies hyperspectral datacubes to obtain particle number, polymer type and size/morphological data in one step. The tools and techniques used in this framework can increase the scalability of sampling MP in aquatic environments. Further development of this framework could enable routine analysis of important ecosystems affected by plastic pollution.

### **3.25.P-We295 A Novel Method for Density Separation Followed by Quantitative $^1\text{H}$ -Nuclear Magnetic Resonance Spectroscopy for the Determination of Microplastics in Marine Sediments**

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Microplastics (MPs), defined as plastic particles smaller than 5 mm, are considered a new class of persistent environmental pollutants. MPs have been found in almost every compartment affected by human interaction but are of particular concern in the marine environment as a major sink of those pollutants. The determination of MPs in the marine environment, particularly sediments and sand, is very laborious. Separation of MPs from other particles is typically conducted in costly large density separators. Then, determination is most often performed by infrared (IR) or Raman spectroscopy, or by pyrolytic methods. Consequently, the development of reliable, simpler, and cost-effective analytical methods is necessary to better assess their distribution in the marine environment at a larger scale.

Therefore, the aim of this study was to develop a method for the separation, identification, and quantification of MPs from aquatic sediments. With that purpose, a density separation using a novel small-scale separation setup made of glass was developed. Thus, it was tested for the separation of sediments (100 g) with spiked with different MPs (polyethylene, polystyrene, polypropylene, polyvinyl chloride and polyethylene terephthalate) at two environmentally realistic concentration levels (2 and 20 mg). The density separation was done with 1.6 g/cm<sup>3</sup> zinc chloride solutions. The trueness of the method was calculated by a gravimetric method and quantitative  $^1\text{H}$  Nuclear Magnetic Resonance spectroscopy (qNMR) for polyethylene terephthalate, polyvinyl chloride and low-density polyethylene, producing recoveries higher than 81% in all cases. Moreover, the combination with qNMR led to limits of quantification below 0.01 ng/g for these three polymers. Finally, the developed method was applied to a few samples of beach sediments from different sites of the Galician coast (Norwest of Spain) where concentrations of polyethylene terephthalate and low-density polyethylene ranged from 62 to 186 ng/g and 82 to 100 ng/g, respectively.

### **3.25.P-We296 Investigation of Polymer Type and Particle Size on Microplastic Enrichment from Sediment Samples**

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Microplastics represent an increasing environmental problem, which is more and more in the focus of the society and politics. To develop strategies and solutions for the targeted prevention of microplastics and for environmental protection, reliable environmental monitoring is very important. For an analysis of environmental samples, an enrichment of microplastic from these samples is absolutely necessary. Without an enrichment of the samples, the limits of detection and quantification cannot be guaranteed. Two things are critical in the enrichment processes. First, no microplastics should be lost in the process. Secondly, the environmental matrix must be separated. The higher the degree of the separated matrix, the simpler and more reliable is the final microplastic analysis. Various factors of the sample itself and during separation are responsible for the success of the enrichment process. In this work, two different polymers in different particle sizes were investigated during electrostatic and density separation. These were the two semi-crystalline thermoplastic polymers LD-PE and PET. These represent two completely different polymers due to their density difference and chemical structure. The polymers were classified into the five fractions < 63  $\mu\text{m}$ , 63 - 125  $\mu\text{m}$ , 125 - 250  $\mu\text{m}$  and 500 - 2000  $\mu\text{m}$  by cryogenic milling and sieving. All particle fractions investigated were tested in a sand well suited for separation with regard to separation success. The success of the processing of the samples was evaluated by the degree of separation on sediment and by a DSC analysis and the calculated recovery rates of the particles. It was found that small and large particles show difficulties in separation. Particles that are too small can be easily lost in electrostatic separation. Particles that are too large are difficult to analyze in the fractions due to sample inhomogeneities. This leads to large value fluctuations and uncertainties in the results. With the particles of medium size, good separations and recoveries can be obtained. Between polymer types, it was observed that the measurement uncertainty for PET is greater than that for LD-PE. The enrichment of the samples with LD-PE was higher than the enrichment of the PET samples. In addition, it was found that multiple separation in electrostatic separation results in a loss of microplastics.

### **3.25.P-We297 Is Polypropylene Relevant for Microplastic Analysis?**

**Maren Meurer**, Yosri Wiesner, Tassilo Waniek, Anja Geburtig and Korinna Altmann, Federal Institute for Materials Research and Testing, Germany

Nowadays, in every terrestrial and aquatic ecosystem, even in the remotest areas, small residues of plastics, the so called microplastic (MP) can be found. MPs are particles with a size of 1-1000  $\mu\text{m}$  (ISO/TR 21960:2020), mainly containing synthetic

polymers like polyethylene (PE), polypropylene (PP), polystyrene (PS) or polyethylene terephthalate (PET). Even styrene-butadiene rubber (SBR) as an indication for tire wear is included due to similar particle formation. To understand the MPs consequences to the environment, it is of high priority to capture its extent of contamination. It is surprising that in the analysis of polymer masses in environmental samples, PE, PS and SBR are often detected, but only small amounts of PP, although this is the second most commonly produced standard plastic and many MP particles originate from carelessly disposed packaging materials. This presentation provides hypotheses about the reasons of rare PP identification and mass quantification in environmental samples. Different investigations of pristine PP and representative environmental samples, including the pre-treatment by Accelerated Solvent Extraction (ASE) or with density separation followed by the thermal extraction / desorption gas chromatography-mass spectrometry (TED-GC/MS) are presented. The results are discussed according to the material properties and a possible degradation mechanism under different weathering conditions which indicate less stability under relevant storage conditions.

### **3.25.P-We298 Considering Both Effect Sizes and Test Concentrations Improves Conclusions Drawn from Meta-analyses in Ecotoxicology**

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The amount of microplastic (MP) particles in the environment is rising, and several studies on the toxic effects of MP particles on biota have been published in the last few years. However, whether MP represents a general risk for ecosystems and which MP traits are associated with adverse effects remains uncertain. Individual studies provide evidence that, for example, MP size, shape, and the presence of an eco-corona may directly affect toxicity. However, there is still much controversy surrounding whether these patterns are generalized for different species, MP trait combinations, and endpoints. To reach more broad conclusions, it is thus necessary to aggregate the available information from different studies in combined analyses. Meta-analyses are a way of quantitatively aggregating results from previous studies. Although meta-analyses have become more broadly used in ecotoxicology, they often focus on the presence/absence of effects (i.e., using concepts based on p-values such as NOEC/LOEC) rather than looking directly at concentration-dependent effect sizes (i.e., dose/concentration-response relationships). We use an example dataset on toxic effects of micro- and nanoplastic particles on *Daphnia* spp. and theoretical considerations to investigate the importance of incorporating concentration-dependent effect relationships in ecotoxicological meta-analyses. To this end, we compare the information we can gain when applying three different meta-analytical approaches: (1) grouping of test concentrations into concentrations leading to significant and non-significant results, (2) looking directly at effect sizes without taking concentration into account (forest plots) and (3) investigating effect sizes in a concentration-dependent manner (regression approach). As concentration is usually a confounder in environmental toxicity studies, we argue that taking both effect sizes and test concentrations into account in ecotoxicological meta-analyses is beneficial to draw reliable conclusions.

### **3.25.V Towards Harmonized Nano- And Microplastics Quantification: Reference Materials, Analytics and Improved Experimental Designs**

#### **3.25.V-01 Microplastics Are Hidden in Sediments: A Case Study from a Natural Protected Area of Guatemala**

*Carlos Mazariegos<sup>1</sup>, Sonia Munoz<sup>1</sup> and Jaclyn Canas-Carrell<sup>2</sup>, (1)Environmental Toxicology, Texas Tech University, USA (2)Dept. of Environmental Toxicology, Texas Tech University, USA* Microplastics (MPS) are an emerging problem for this century threatening human health and the environment. MPS are cosmopolitan particles, however undeveloped countries are more vulnerable to MPS pollution due to their lack of environmental rules, waste management, and indiscriminate consumption of every type of plastic material. Guatemala has three protected areas on the south coast intended to support biodiversity and natural services for communities. The Reserva Natural de Usos Múltiples Monterrico is recognized for having biodiversity including mangroves, fish, birds, and flora. The area is economically important for small-scale fisheries. However, little is known about MPS contamination in water, sediments, fauna, and flora in this area. Thus, the purpose of this study was to assess MPS accumulation and distribution in sediments from the Reserva Natural de Usos Múltiples Monterrico. Sediments were collected using a trawl from six places along the estuarine system classified as non-populated and populated. Then, MPS were separated by using 1- and 5-mm steel sieves. Finally, MPS were counted, classified according to shape, and identified using an infrared spectroscopy technique (MPS polymer identification is ongoing). 344 MPS were registered, and film and fragments were the predominant shapes found. No differences were detected between non-populated and populated sites ( $p > 0.05$ ). Results from this work will allow an understanding of MPS accumulation and distribution in sediments from an important protected area of Guatemala. Additionally, the information might be useful for stakeholders to inform the advances of the UN's sustainable development goal number 14: life below water.

#### **3.25.V-02 Size Limitations on the Data-driven Identification of Microplastic Using FD-FLIM**

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Currently, a fast and reliable ecological risk assessment is time-consuming due to the challenging purification which has to be done to analyze the microplastic (MP) samples taken from the environment using the established analytical methods. Thus, an urgent need exists concerning a fast and reliable identification of plastic-type, shape and size. Recent studies on Frequency-Domain Fluorescence Lifetime Imaging Microscopy (FD-FLIM) show high potential to quickly identify the plastic type, shape and size and differentiate plastics from environmental materials. However, it has yet to be tested down to which size MP samples



can be identified using FD-FLIM. To determine the identification limitations, LDPE, PET, PP and PS spiked with 0.012 wt% of fluoresceine are prepared in 1000 x 1000 x 20  $\mu\text{m}$  down to 125 x 125 x 20  $\mu\text{m}$  quadratic particles. Six particles of each size are placed next to each other on microscope slides and sealed with coverslips. The experimental setup consists of a modulated laser, an FD-FLIM camera, a microscope (objective: x20, imaging field: 0.28 mm x 0.28 mm), and two optical filters. Twelve FD-FLIM measurements are done on 1 mm particle of spiked LDPE, PET, PP, and PS MP particles and evaluated by Gaussian analysis. The resulting expectation value and standard deviation of the FLT serve as thresholds. Combined with an image processing algorithm consisting of gaussian blur, binarization and erosion, we set the minimum detectable particle size to 125  $\mu\text{m}$ . With this, identifying the plastic type, shape, and size was possible for 6/6 images of the 125  $\mu\text{m}$  particles from LDPE, PP and PS and 3/6 images from ABS. However, comparing the results of the FLT of the fluorescein-spiked particles with the FLT of pure PET and PP, a decrease in FLT is obtained for PET (-1.2 ns) and PP (-0.5 ns), while the FLT of LDPE and PS are within one standard deviation of the FLT of the pure LDPE and PS.

In conclusion, fast and reliable identification of the plastic type, shape and size is possible down to a size of 125  $\mu\text{m}$ . Hence, further studies with particles smaller than 125  $\mu\text{m}$  must be conducted to set a detection limit. Nevertheless, the additive concentration impacted two plastic types, which may lead to difficulties in the identification. Therefore, a heuristic model has to be investigated to determine how plastic containing different additive concentrations can be identified by their characteristic FLT.

### 3.25.V-03 Analytical Alternatives to Detect and Quantify Nanoplastics in Complex Environmental Matrices

**Patricia Taladriz-Blanco**<sup>1</sup>, Ana Castanheira<sup>2</sup>, Monica Quarato<sup>2</sup>, Alexandre Chicharo<sup>3</sup>, Sara Abalde-Cela<sup>3</sup>, Begoña Espina<sup>4</sup> and Laura Rodriguez-Lorenzo<sup>2</sup>, (1)Water Quality, International Iberian Nanotechnology Laboratory, Portugal, (2)Water Quality Group, International Iberian Nanotechnology Laboratory (INL), Portugal, (3)Medical Devices Group, International Iberian Nanotechnology Laboratory (INL), Portugal, (4)International Iberian Nanotechnology Laboratory, Portugal

Detecting and quantifying nanoplastic particles (< 1000 nm) at environmentally relevant concentrations (ng/L - ug/L) using conventional analytical techniques such as Raman and Infrared spectroscopy (IR) or Fluorescence microscopy (FM) represents one of the biggest challenges for this research field. The main challenge relies on the instrument size, which for confocal Raman is  $\geq 0.25 \mu\text{m}$  (lateral), FM is  $> 1 \mu\text{m}$  and FTIR  $> 2\text{-}5 \mu\text{m}$ . Additional challenges related to their low concentration in natural environments, the complexity of the sample matrices, their ubiquity, and their inherent physicochemical properties raise the need to develop and standardise analytical methods for nanoplastic particles. Herein, we describe the work done in our research group on developing different approaches based on Surface-Enhanced Raman Spectroscopy (SERS), Nanoparticle Tracking Analysis (NTA), and electron microscopy (SEM and TEM) to fulfil the existing gap in analytical techniques to detect and quantify nanoplastic particles.

### 3.25.V-04 Nanoparticle Tracking Analysis to Quantify Nanoplastics in Complex Environmental Matrices

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Detecting and quantifying nanoplastic particles (< 1000 nm) at environmentally relevant concentrations (ng/L - ug/L) using conventional analytical techniques represents one of the biggest challenges for this research field. Often, a pre-treatment step, including pre-concentration and removal of undesirable material, is required. Cloud point extraction (CPE), using a non-ionic surfactant such as Triton-X, has been shown to successfully separate and concentrate nanoplastics in complex environments. Nanoparticle tracking analysis (NTA) relies on scattering properties and is used to quantify and calculate nanoparticle size (distribution). Compared to dynamic light scattering, NTA has the advantage that the results are calculated as number-weighted; therefore, size values are less biased by the presence of aggregates or larger nanoparticles. NTA is a non-destructive technique, representing a promising tool for analysing nanoplastic particles compared to the mainly used Pyrolysis Gas Chromatography-Mass Spectrometry. Herein, we show the combination of CPE and NTA to successfully quantify and characterise nanoplastics (100 and 200 nm – Polystyrene) in natural fresh and salt waters. 50 nm – gold and 100 nm – silver nanoparticles were used as a control due to the better optical properties compared to nanoplastics for a defined size.

### 3.25.V-05 Surface Enhanced Raman Scattering for Rapid Monitoring of Microplastics

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Given the current circumstances of widespread use of plastic products and lack of means to deal with them, microplastics (MPs), as an emerging contaminant, has become a global concern. Many studies have demonstrated that microplastics can be found in numerous environmental samples. The widespread distribution of microplastics poses a threat to the biological community, but the toxicology of microplastics has not been adequately studied. The most commonly used methods for chemical and physical identification of microplastics are Fourier transform infrared spectroscopy (FTIR) and Raman. However, these two methods are limited by poor spatial resolution, which is 20  $\mu\text{m}$  and 5  $\mu\text{m}$  respectively. Surface-enhanced Raman scattering (SERS) can significantly enhance the weak Raman signal, thus making it a promising tool for microplastics detection at a low concentration. In this work, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) are employed as enhancement substrate to enhance the Raman signal of microplastics. The experimental conditions were optimized to achieve better analytical performance, including the size of the nanoparticles, the amount of addition, the ionic strength of the solution and the ratio of nanoparticles to microplastic particles. To investigate the effect of AuNP size on Raman signal enhancement, three size AuNPs are tested. To determine the appropriate amount of gold nanoparticles for microplastics, different amounts of the gold nanoparticles were tested. Results show that AuNPs can significantly enhance the Raman signal of microplastics. A strong relationship between the size of

AuNPs and the enhancement performance is identified in this work. This platform has the potential to be a more sensitive microplastic detection approach and serve as a new tool for rapid monitoring the spatial and temporal distribution, toxicity, and environmental exposure of microplastics.

## Track 4: Ecological and Human Health Risk Assessment of Chemicals, Mixtures and Stressors and Risk Mitigation Strategies

### 4.01 Advances in Environmental Risk Assessment of Chemicals

#### 4.01.T-01 National Risk Trends Based on Pesticide Sale Data in Germany - A Comparison of Five Indicators

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The reduction of environmental and health risks from the use of pesticides in agriculture is a corner stone of the recent European Farm-to-Fork Strategy of the European Commission, calling for a 50% reduction in the amount and risks of pesticides by 2030. EU policies like the Sustainable Use Directive (Directive 128/2009/EC) already demand the implementation of National Action Plans to reduce environmental risks from pesticide use.

To monitor the ubiquitous efforts towards risk reduction, a robust assessment and indicator system is required. Thus, comprehensive and transparent indicators are needed to capture, on national level, the heterogeneity of active ingredients in terms of their potential impact on the environment and human health. The Harmonized Risk Indicator (HRI) was developed as a harmonized approach for all EU Member States to assess the risk trends on national level and EU level to represent the risk caused by pesticide applications. Such an approach requires EU-wide availability of the necessary data as well as an appropriate degree of complexity in the calculation of the indicator. To meet these requirements, the COM has adopted the HRI, which combines the hazard classification under Regulation 1107/2009 with the sales statistics.

To reflect the risk trend of AIs, the assessment must take into account their intrinsic properties and specific toxicity values. Several existing risk indicators follow this approach. Numerical values are assigned according to the various AI properties and toxicity end points and added for several AI properties to produce an AI-specific weighting factor that reflects toxicity to humans and the environment, as well as environmental fate. The Environmental Impact Quotient and Toxic Load Indicator were developed based on such an approach. In Denmark, the Pesticide Load Indicator, has been used to estimate risk at national and regional levels and to calculate pesticide taxes. In Sweden, the Pesticide Risk Indicator was developed to map long-term risk trends at the national level.

To compare the trend development of the different indicators, we calculate all indicators with the German sales data and present the results in the Pesticide-Trend Database Explorer (<https://sf.julius-kuehn.de/pesticide-dbx/>), a transparent online tool for flexible evaluations of the indicators regarding the base line period, the weighting factors and the different AI groups. Based on these results simple improvement of the HRI are discussed.

#### 4.01.T-02 Validating Predicted No Effect Concentrations (PNECs) in the Field

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Both the derivation of predicted environmental concentrations (PECs) and predicted no effect concentrations (PNECs) used in the environmental risk assessment of chemicals rely on various models and assumptions. Particularly for PNECs, a validation of these models and assumptions in the field is pending and challenging as targeted post-authorisation monitoring is scarce and specific ecological effects are largely masked by other influences. In this exemplary assessment we aim to answer the questions what steps enable a validation of PNECs, whether the PNECs for pesticides we monitored were adequate and of what use such a field-based validation can be. On the basis of a large stream monitoring data set of >100 small streams we linked chemical (75 pesticides in >800 water samples) with biological measurements (invertebrate communities) to validate the PNECs of primarily invertebrate-toxic pesticides in surface waters.

We identified five general steps to be performed to validate PNECs with field data: 1) Measure environmental exposure adequately. 2) Assess a suitable ecological response. 3) Link dose and response. 4) Define a protection. 5) Integrate uncertainty. Referring to our case study, we were able to link the ecological effects observed reflected by the invertebrate-based and pesticide-specific SPEcies At Risk (SPEAR<sub>pesticides</sub>) indicator to the maximum single pesticide-related factor of PNEC-exceedance measured using a linear correlation ( $R^2 = 0.44$ ,  $p < 0.001$ ). We found that, in the average stream subjected to various chemical and environmental stressors, peak concentrations equalling the PNEC were already associated with a SPEAR<sub>pesticides</sub> that did not comply with the previously defined protection goal.

Our analysis questions the established principles, data requirements and assessment factors according to which pesticide PNECs for surface waters specifically and PNECs in general are derived. The use of this field-based validation and respective consequences for pesticide regulation are to be discussed.

#### 4.01.T-03 Development of Analytical Frameworks to Assess the Risks Posed to Soil by Emerging Contaminants

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Many unregulated contaminants, often termed Emerging Contaminants (ECs), are globally released on soil creating potential risks, especially when undetected leading to significant impact on environmental receptors. The UK ministry of Defence plays an important role in soil protection as the biggest holder of Sites of Special Scientific Interest (SSSI) in the UK as well as being an active polluter in these protected areas through essential training activities. Therefore, improved methods for early identification of emerging contamination are needed to avoid long term environmental impacts and costly remediation. This research has contributed to the development of a soil analytical framework to facilitate early identification of the deleterious effects of ECs and chemicals of military concern. Two different scenarios were considered, firstly, when there is a significant body of ecotoxicological data available for a specific contaminant in the literature and secondly, when ecotoxicological data is not available.

Firstly, a hazard-base scale was developed and designed to categorise chemicals into low, medium, and high environmental hazards to provide an early indicator for soil degradation at low-cost. This research showed that for the contaminants of interest (e.g., hexahydro-1,3,5-trinitro-1,3,5-triazine, trinitrotoluene, perfluoroalkyl, polyfluoroalkyl and Cypermethrin) the low-level hazard values were lower than expected when compared to Soil Screening Values (SSVs) and Ecological Soil Screening Levels (Eco-SSL). This means the contaminants are likely to have a negative impact on the soil at lower concentrations. Secondly, Insensitive High Explosive (IHE) compositions were identified as lacking data and so soil mesocosms were carried out to define the long-term consequences on soil. Field experiments were used to quantify IHE residue deposition concentrations from a standard 155 mm artillery shell, which was then used to estimate low, medium, and high contaminant concentration for soil mesocosm studies. Mesocosms were used to quantify the impact on soil using indicators identified through literature review. Specifically, this work showed that the frequent use of IHE filled munitions on training ranges influence the quality of the soil even when low quantities of energetic residue are deposited. Results from this work are a first step towards a more comprehensive soil analytical framework development providing early identification tools for soil protection.

#### **4.01.T-04 Effect of PAC (PAH & Polar-PAC) Availability on Terrestrial Organisms' Ecotoxicity**

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Historically contaminated sites have gained attention in recent decades. Some of them, e.g., former coking plants are contaminated by polycyclic aromatic compounds including PAHs and polar-PACs (O/N/S-PACs). The exposure of terrestrial organisms to freshly contaminated soil with PAC demonstrated significant toxicity towards organisms. However, for some soils sampled from historically contaminated sites exhibiting a higher PAC content, tests performed on earthworms have shown a limited impact on mortality rates.

Based on studies, in historically contaminated sites, PAC (bio)availability is generally limited. This phenomenon known as "aging" is a main factor to consider in the evaluation of toxicity risk in soil contaminated by PAC.

In fact, for a refined estimation of the environmental risk and understanding of the impact of the PAC (bio)availability on toxicity, it's necessary to combine a complete chemical characterization (total content and available fraction of PACs) and biological expositions.

This work aims to evaluate the toxicity of two aged coking plant soils by running bioassays on invertebrates and plants. The toxicity of these soils (low PAC availability) will be compared with the same soils previously treated (heating) to increase their PAC availability.

A pre-heating treatment was carried out to increase PAC availability, then the soil was extracted with dichloromethane (DCM) and the PACs (i.e., PAHs and O/N/S PACs) were quantified by GC-MS. As a second step, ecotoxicity tests were performed through limit assays using acute bioassays. The purpose of this step is to evaluate the earthworm viability rate and seed development of plants (seedling emergence and growth) and to highlight the importance of taking into account the availability factor.

Results of this study showed that soils with identical physico-chemical properties but exhibiting contrasted levels of PAC availability induced different ecotoxic responses of terrestrial organisms, an increased availability leading to a much higher toxicity. These results pointed out the importance of considering the contamination availability parameter in risk assessment and not only the total contaminant concentration.

#### **4.01.T-05 Chronic Toxicity and Bioaccumulation Testing of Heterocyclic Polyaromatic Hydrocarbons with *Daphnia magna* – Controlling Exposure with Passive Dosing**

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Heterocyclic polyaromatic hydrocarbons (heterocyclic PAHs) substituted with sulphur, nitrogen, or oxygen heteroatoms are often released along with their homocyclic equivalents but are often present in higher concentrations due to their higher polarity.

Heterocyclic PAH are often toxic and persistent in the aquatic environment and can pose a threat to aquatic life. Nevertheless, reliable toxicity data, especially for chronic exposure, are still very limited. Given the "difficult" nature of heterocyclic PAHs, i.e., poor water solubility and high hydrophobicity, conducting toxicity test using standard approaches is very challenging and not always meaningful. Compound losses often occur, e.g., through sorption during the test, resulting in an unstable exposure. Passive dosing can overcome these challenges by maintaining a defined and stable exposure during the test. Although passive dosing method was used efficiently for short-term toxicity testing, its applicability for long-term toxicity tests (21-days), where the exposure medium has to be renewed and test organisms have to be fed, was so far not performed. In this study, we employed the passive dosing method in the chronic toxicity test with *Daphnia magna* to assess heterocyclic PAHs. To maximize the information output, the chronic toxicity test simultaneously served as an uptake phase of the bioaccumulation test, which was

subsequently followed by the depuration phase. The adaptability and effectiveness of using this method in routine toxicity testing is critically debated.

#### 4.01.P Advances in Environmental Risk Assessment of Chemicals

##### 4.01.P-Mo261 A Pulsed Exposure Approach to Investigate Chronic Fish Developmental Stage Mortality and Its Relevance for Risk Assessment

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The chronic toxicity of pesticides to fish is typically evaluated using standard methods e.g., OECD 210, Fish, Early Life Stage toxicity (FELS). In addition, OPPTS 850.1500 guidance describes the method to conduct the fish life cycle (FLC) test that can be used to detect adverse effects on development, growth and reproduction over an entire life cycle. In some cases, effect endpoints reported in FELS and FLC studies can be inconsistent and further investigation of development growth stage effects may be justified.

In this case, we will present findings from a real example where development growth stage specific effects were reported from a FLC study that were inconsistent with findings from other chronic fish studies with the same test substance.

We intend to share lessons learned of the practicalities in delivering a chronic fish GLP study using pulse exposure scenarios to different life stages. This includes using both semi-static and flow-through exposures, and the brief use of a prophylactic egg fungicide. We will also present findings from supporting chemical analysis to characterise the exposure pulses and present recommendations for the consideration of fish developmental stage toxicity in pesticide regulatory risk assessment.

##### 4.01.P-Mo262 Graphical Matching of Exposure Profiles in Pulsed Exposure Toxicity Tests to Predicted Environmental Concentrations – A Helpful Tool in Higher-Tier Aquatic Pesticide Risk Assessments

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Following the current EFSA Aquatic Guidance Document (AGD), the risks for aquatic organisms can inter alia be refined by performing pulsed (peak) exposure tests or mesocosm studies in the tiered risk assessment scheme. The endpoints of these studies (NOEC, EC<sub>x</sub>) can be used together with an assessment factor to define a regulatory acceptable concentration (RAC). In risk assessments, this RAC is compared to the maximum predicted environmental concentration (PEC<sub>max</sub>). However, the question whether the exposure in higher-tier studies covers the predicted exposure profiles (e.g. FOCUS scenarios) has gained considerable relevance in recent years.

The reason is that environmental exposure is temporally variable in aquatic environments, with peak exposures after application due to drift, followed by run-off or drainage events to respective water bodies. The shape of this temporal exposure profile influences uptake and effect of compounds. Consecutive exposure peaks might be ecotoxicologically dependent leading to an accumulation of internal concentrations and possibly additive effects. To demonstrate that exposure regimes in higher-tier studies adequately cover predicted consecutive environmental exposure peaks in a worst-case scenario, a comparison of the exposure profile in the higher-tier study to the predicted exposure profile is required. However, due to the variety of exposure scenarios included in the FOCUS model, a single worst-case scenario often cannot be identified.

We present a practical tool programmed in statistics software “R”, which expands EPAT functionality to compare the time course of exposure profiles of higher-tier experiments with predicted exposure profiles from FOCUS scenarios. In brief, we illustrate the three application steps: (1) Using a moving time window to scan the predicted exposure profile for critically high exposure peaks; (2) Exposure profiles of the higher-tier study are graphically overlaid to the environmentally predicted profiles; (3) Both exposure patterns are compared visually by the user.

The outcome of this approach supports the definition of the regulatory acceptable profile (RAP). Advances, shortcomings, and potential further adaptations of the approach are discussed.

##### 4.01.P-Mo263 Heterogeneity in Biological Assemblages and Exposure in Chemical Risk Assessment: Exploring Capabilities and Challenges in Methodology with Two Landscape-Scale Case Studies

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Chemical exposure concentrations and the composition of ecological species vary in space and time, resulting in landscape-scale heterogeneity. Current regulatory, prospective chemical risk assessment frameworks do not directly address this heterogeneity because they assume that reasonably worst-case chemical exposure concentrations co-occur (spatially and temporally) with species that are the most sensitive to the chemical's toxicity. Whilst these approaches aim to be protective, a more precise understanding of when and where chemical exposure and species sensitivity co-occur enables risk assessments to be better tailored and applied mitigation to be more efficient. We used two aquatic case studies covering different spatial and temporal resolution to explore how geo-referenced data and spatial tools might be used to account for landscape heterogeneity of chemical exposure and ecological assemblages in prospective risk assessment. Each case study followed a stepwise approach: i) estimate and establish spatial chemical exposure distributions using local environmental information and environmental fate models; ii) derive toxicity thresholds for different taxonomic groups and determine geo-referenced distributions of exposure-toxicity ratios, i.e., potential risk; and iii) overlay risk data with the ecological status of biomonitoring sites to determine if relationships exist. We focus on demonstrating whether the integration of relevant data and potential approaches is feasible rather than making

comprehensive and refined risk assessments of specific chemicals. The case studies indicate that geo-referenced predicted environmental concentration estimations can be achieved with available data, models and tools but establishing the distribution of species assemblages is reliant on the availability of a few sources of biomonitoring data and tools. Linking large sets of geo-referenced exposure and biomonitoring data is feasible but assessment of risk will often be limited by the availability of ecotoxicity data. The studies highlight the important influence that choices for aggregating data and for the selection of statistical metrics have on assessing and interpreting risk at different spatial scales and patterns of distribution within the landscape. Finally, we discuss approaches and development needs that could help to address environmental heterogeneity in chemical risk assessment.

#### **4.01.P-Mo264 If The 95th Percentile Risk Quotient of a Substance in Surface Waters in a Country Is Above 1 Is It Always a Country-wide Risk?**

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When more than 5 % of sites within a country have a risk quotient (RQ) above 1 for a substance in many jurisdictions this is concluded to be a country-wide risk. In the European Union if there are 4 or more countries meeting this criteria it is subsequently concluded that the substance is a continent wide risk. During the evaluation of the criteria there is, typically, no consideration of the location or characteristics of sites that are exceeding the RQ threshold of 1.

The aim of this poster is to highlight that the location of the exceeding sites should also be accounted for when using this metric. Local, or regional, risks may incorrectly be assigned as nationwide risks if the spatial extent is not considered. This could then potentially lead to a substance being concluded as a continental-wide risk when the exceedances are located in specific regions of specific countries. This is of particular importance for natural occurring substances that will be greatly impacted by natural geologic factors.

This poster will use examples where the metric does show that the substance does pose a nation-wide risk due to greater than 5 % of sites having an RQ above 1 as well as examples of where although greater than 5 % of sites are exceedances, they are all (or predominantly) limited to a specific area. Through these examples it can be shown that the 5 % of sites exceeding criteria is not in itself a poor estimate of risk, but that it should not be used in isolation to determine the risk of a substance within a country.

#### **4.01.P-Mo265 Mechanism-based Prioritization of Chemicals using ToxCast™ Database: A Case Study with Priority Existing Chemicals under K-REACH**

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A new chemical regulation in Korea, the Act on Registration and Evaluation of Chemicals (K-REACH) came into force on January 1, 2015. However, the toxicity data required to register and evaluate the chemicals is insufficient. Use of high-throughput in vitro bioactivity data in setting prioritization of chemicals provide a new feasibility to inform mechanism-based screening. Herein, we conducted a systematic analysis on 510 chemicals, priority existing chemicals regulated under K-REACH, using 949 ToxCast bioassays with intended target genes to identify the potential toxicity mechanism of the chemicals. First, we computed a comprehensive hit-call data matrix contained 298984 chemical-gene integrations. Based on the reactivity to the chemicals, we selected top 20 bioassays whose intended target genes' families were CYP, oxidoreductase, transporter, nuclear receptor, steroid hormone, GPCR, DNA binding. We also selected top 20 chemicals based on the reactivity to the bioassays. They are mainly in consumer products including colorant, surfactant, and detergent. Further, using set analyzer tool provided in Comparative Toxicogenomics Database (CTD), we investigated the enriched pathways and diseases of intended target genes active with top 20 chemicals. The enrichment analysis showed these genes may play diverse roles in cell differentiation/development, proliferation, and metabolism and induce various diseases including pathologic processes (MESH:D010335), cardiovascular diseases (MESH:D002318), skin and connective tissue diseases (MESH:D017437), and chemically-induced disorders (MESH:D064419). Our result demonstrates that ToxCast™ bioassay data can be used for mechanism-based chemical categorization. Further study on chemical prioritization using adverse outcome pathway (AOP) by predicting in vitro target gene activity data in ToxCast is in progress.

#### **4.01.P-Mo266 High-Content Screening of Polycyclic Aromatic Hydrocarbons (PAHs) by the Cell Painting Assay: Harnessing Cell Phenotypes for the Risk Assessment of Chemicals**

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Polycyclic aromatic hydrocarbons (PAHs) are widespread across the globe mainly due to long-term anthropogenic sources of pollution. The inherent properties of PAHs such as hydrophobicity, and thermostability have made them highly persistent in the environment. PAHs have been found highly toxic, mutagenic, carcinogenic, and teratogenic to various life forms. Even though hundreds of PAHs exist, risk assessments of PAHs in contaminated environmental samples are usually based on instrumental quantification of a small number of individual PAHs. The next-generation blueprint of toxicology at the U.S. Environmental Protection Agency (EPA), (i.e., USEPA CompTox Blueprint), advocates the use of nontargeted, high-throughput profiling assays for initial characterization of the biological activity of environmental chemicals, including PAHs. Such profiling assays should be capable of being deployed in a high-throughput, concentration-response screening format; and provide high-content data that can be used to identify potency thresholds for perturbation of cellular biology and provide information on putative mechanisms of toxicity. Cell Painting is high-throughput and high-content phenotypic profiling assay that reveals impact of perturbation(s) on eight broadly relevant cell compartments, including nucleus, actin/Golgi/plasma membrane, mitochondria, endoplasmic reticulum, and RNA/nucleoli. In this study, we have exposed human osteosarcoma U-2 OS cells to 20 native, alkylated, oxygenated and OH-PAHs for 24 h using a range of concentrations. Afterwards, cells were fixed, stained following the Cell

Painting protocol and imaged on a high-throughput imaging platform InCell 2200 HTS system (GE Healthcare). Image processing was performed via CellProfiler software (v. 4.2.1; [www.cellprofiler.org](http://www.cellprofiler.org)) using a combination of cell segmentation and defining of cellular compartments. CellProfiler measured ~3.200 morphological features of single cells related to size, granularity, shape, texture, intensity, radial distribution etc. For example, following the exposure we have observed a number of PAH-specific phenotypic profiles, including cytoskeleton alterations, mitochondrial feature changes and micronucleus formation. Employing Cell Painting to study PAHs toxicity, will provide efficient high-throughput *in vitro* methodologies needed to group and prioritize PAHs based on the potency thresholds.

#### 4.01.P-Mo267 Screening the Effects of Coumaphos, an Organophosphate Pesticide on Freshwater Planarian Through Standardized and Reproducible Quantitative Approaches

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Environmental pollution is a worldwide issue. Pesticides are widely used in agriculture and exposure to some can result in adverse effect on the nervous system of animals, especially on non-target organisms. In ecotoxicology, the adoption of the 3Rs rule - reduce, refine, replace - aiming to limit the use of vertebrate animals in scientific research, has precipitated the development of new models using invertebrate organisms for bioassays.

Freshwater planarian, an invertebrate flatworm, have shown to be a promising alternative model to assess chemicals that may end up in aquatic environment. They are worldwide distributed, easy to collect and to maintain in lab. Their small size allows medium to high-throughput *in vivo* screening of toxicity. Planarian can enable the evaluation of toxicity of chemicals on many levels: 1) they are non-target organism; 2) almost all species are endemic to their geographical location; 3) they are good indicators of chemical risk on the aquatic ecosystem; 4) their nervous system share the same neuronal subpopulations and neurotransmitters as the mammalian brain, making them potential screening tools for predicting pollutant toxicity for mammals.

Using a standardized, reproducible quantitative protocol, we assessed the effect of Coumaphos, an organophosphate pesticide on freshwater planarians.

Planarian were screened for mortality over a 24 h period. Effects of sublethal concentrations of Coumaphos were screened through quantitative assessment of multiple behaviour. Endpoints measured includes exploratory behaviour and response to light stress. These tests have shown to be reliable and repeatable for toxicological screening in lab. Coumaphos has never been tested on planarians, nevertheless planarians were more sensitive to Coumaphos than the aquatic arthropod *Artemia salina* with 24h LC<sub>50</sub> of 2.56 M and 585 M (21.23mg/L), respectively (S nchez-Fort n et al., 1995). As it has been demonstrated for other aquatic organisms, exposure to increasing concentrations of Coumaphos results in significant impairment of planarian locomotion, but did not impair response to light stimulation. In planarians, locomotion is a mix of muscular contractions controlled by the cholinergic system, and ciliary beating. Thus a conservation of the negative effect of the organophosphate pesticide on planarian's brain could explain the observed locomotion impairment.

#### 4.01.P-Mo268 Determining the Toxicity of Organic Compounds on the Nematode *Caenorhabditis elegans* based on Aqueous Concentrations

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*Caenorhabditis elegans* is used as a test organism for assessing the toxicity of chemicals in aqueous medium. In the test system, however, chemicals can absorb to the bacterial food source, which reduces the freely dissolved concentrations of the tested compounds. This makes it difficult to compare the toxicity to other test organisms when comparing based on total or nominal concentrations. In this study, *C. elegans* was exposed to seven organic chemicals with varying hydrophobicity, thus also different affinities to bind to the food of *C. elegans*. Measured concentrations of the dissolved aqueous and the bacterial-bound fraction, allowed the calculation of binding constants ( $K_b$ ), that were comparable to literature data of hydrophobic chemicals and correlated well with their hydrophobicity, expressed as log  $K_{OW}$ . The chronic toxicity of the various compounds on *C. elegans*' reproduction, based on their aqueous concentration, was weakly related to their log  $K_{OW}$ , as not all tested substances could unequivocally be classified as narcotic chemicals. The analysis of toxicity on a chemical activity basis suggested baseline toxicity of most compounds, with some chemicals less inert or even specifically acting when comparing to a *C. elegans* specific baseline toxicity model. Compared to the toxicity for *Daphnia magna*, good correspondence of these two test systems in terms of the toxicity ranking of the seven tested chemicals was found, although *C. elegans* showed to be slightly less susceptible. It could be shown that aqueous concentrations in the nematode test system corresponded very well with freely dissolved concentrations that were modelled using partitioning coefficients for food bacteria  $K_b$ , hydrophobicity constants ( $K_{OW}$ ) or a simple mass-balance model from nominal concentrations. This offers the possibility to estimate freely dissolved concentrations of chemicals from nominal concentrations, making routine testing of chemicals more accurate.

#### 4.01.P-Mo269 Nematode Community of a Natural Grassland Responds Sensitively to the Broad-spectrum Fungicide Mancozeb in Soil Microcosms

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Fungicides make up the largest part of the total pesticide use, with the dithiocarbamate mancozeb being widely applied as a non-systemic contact pesticide to protect a wide range of field crops against fungal diseases. Although nematodes are key drivers of soil functioning, data on effects of fungicides, and especially mancozeb, on these non-target organisms are scarce. Therefore, the effects of mancozeb on a soil nematode community from a natural grassland was assessed in small-scale soil microcosms.

Nematodes were exposed to mancozeb-spiked soil in six nominal concentrations (7 – 133 mg/kg dry soil) and analyzed after 14, 56 and 84 days in terms of densities, genus composition and functional traits. As this fungicide is known to quickly degrade in soils (DT50 < 1 day), mancozeb concentrations were analyzed for all sampling occasions. Chemical analysis revealed considerably lower measured concentrations as compared to the aimed nominal soil concentrations at the begin of the exposure (1 to 18 mg/kg dry soil), suggesting a fast degradation during the spiking process. Nevertheless, the native nematode community responded sensitively to the fungicide mancozeb, revealing lower NOEC and EC10 values than reported for other soil invertebrates such as springtails and earthworms. Using the EC10 for the most sensitive nematode community endpoint (% predators and omnivores: 1.2 mg/kg dry soil), the risk assessment exhibited a toxicity exposure ratio of 0.66 and thus a high risk of mancozeb for soil nematodes. Keeping in mind their abundance and their central roles in soil food web functioning, the demonstrated sensitivity to a widely applied fungicide underscores the relevance of the inclusion of nematodes into routine risk assessment programs for pesticides.

#### **4.01.P-Mo270 All of a Flutter: Heart-rate Effects in Response to Diclofenac in Adult (Female) *Moina macrocopa* Strauss 1820**

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The European Union has taken steps to highlight pharmaceutical pollution in the aquatic environment. Successive ‘watchlists’ have targeted an increasingly diverse range of pharmaceuticals found in ground, drinking, influent & effluent waste- waters. One such compound is ‘diclofenac’ (2-[2-(2,6-dichloroanilino)phenyl]acetic acid; CAS ID: 15307-86-5)(DCF). DCF was listed in European Commission Decision 2015/495 on March 20, 2015 in the water watchlist under the Water Framework Directive (WFD). The WFD is a mechanism for obtaining high-quality European Union-wide monitoring data on potential water pollutants for the purpose of determining the risk they pose and thus whether Environmental Quality Standards (EQS) should be set for them at EU level. According to the EQS Directive (article 8b), this list should be updated every 2 years.

Therefore, rather than detecting the presence of, and recording the levels of DCF in waters, it is incumbent upon researchers in the field to determine specific effects on living organisms. To ascertain in the first instance, a gross metabolic effect as a result of the presence of DCF in water in which organisms live we investigated the relationship between concentrations of DCF and the heart-rate of adult (female) *Moina macrocopa* Strauss 1820. *Macrocopa* is a homologue of *Daphnia* spp., and consists of two sub-species - one prevalent in the Americas, and the other in Eurasia - and the classic heart-rate protocol applies well to this species. The *M. macrocopa* were acutely exposed to environmentally relevant DCF concentrations and were studied using the classical ‘heart-rate’ protocol as is well known for *Daphnia* spp. as an intended non-lethal method of investigation.

*M. macrocopa* were observed *in vivo* in cavity slides under short spans under a microscope with a smart-phone attachment. Recordings were taken of the heart-rate and on slowing to an easily observable rate, beats were counted using a hand counter. The chronic effects of the pharmaceutical were then established by observing organ function and we report the effect of DCF on the rate and rhythm of the crustacean ‘heart’ as a function of overall life processes. This work is part of a larger project examining the acute and chronic effects of a range of aquatic pharmaceuticals on a range of macroinvertebrates.

#### **4.01.P-Mo271 Ecotoxicity of Water-Soluble Synthetic Film**

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Water-soluble synthetic film and polymer (WSSP) are used in industrial, food, household and biomedical fields, but their impact in the aquatic environment and effects to aquatic organisms are still unclear.

To clarify their impact to aquatic environment, two grades of water-soluble films (Hi-Selon™ from Mitsubishi Chemical Corporation) made from polyvinyl alcohol which is one of the typical WSSPs and used as water-soluble packaging of liquid detergent, and 5 kinds of additives in those films were investigated by acute and extended-acute toxicity test using water flea (*D. magna*). And fry of medaka (*O. latipes*) were used in acute toxicity test.

As the result, no acute toxicity was observed with both *D. magna* and *O. latipes* even at a concentration of 1000 mg/L which is 10 times higher than the maximum concentration commonly used in toxicity test. In an additional experiment of acute toxicity test with *D. magna* under 100 times higher concentration, the swimming inhibition was observed. This result is considered probably due to the physical (viscosity/osmotic pressure) effects of the substance.

Although some of the additives showed weak toxic effects on *D. magna*, the amount of the additives contained in the film were less than 1% by weight. Their dissolutions from the film into the environment is expected to be too small, thus their impact is thought to be negligible.

Based on our current understanding, it is thought that water-soluble film Hi-Selon™ is ecologically safe.

#### **4.01.P-Mo273 Ecotoxicity of Hydroquinone on Non-target Soil and Water organisms**

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Hydroquinone (HQ) is a metabolite of benzene that is ubiquitous in the environment due to its use in industrial activities and as a human topical treatment. It is present in many natural sources, such as various plants, and can also be produced by chemical synthesis. Industrially, it can be used as a stabilizer in paints or varnishes, as a colorant and as a developing agent in photography. As a cosmetic formulation, it is used to treat hyperpigmentation, although the safety of exposure is still unclear.

The aim of this study was to determine the ecotoxicological effects of HQ. *Allium cepa* and *Eisenia foetida* were used as biological indicators of terrestrial environments, while *Daphnia magna* and *Vibrio fischeri* were used as indicators of aquatic environments.

*A. cepa* was exposed to 0.03, 0.3, 3, 30, and 300 µg/mL of the compound and the impact on root inhibition was measured after a 72 hours incubation as an endpoint. HQ showed phytotoxic effects with EC<sub>50</sub> values = 7.631 (6.720-8.676) µg/mL.

Adult individuals of *E. foetida* were placed for 14 days in soil containing 0.2, 2, 20, 200, and 2000 mg/Kg of HQ. After the exposure time, the number of dead worms was measured, resulting in an LC<sub>50</sub> higher than 140 mg/Kg.

The acute toxicity of HQ in *D. magna* was studied by exposing these aquatic invertebrates to 0.01, 0.1, 0.5, 1 and 2 µg/mL and measuring their mobility after 24 hours, showing a value of EC<sub>50</sub>=0.142 (0.104-0.204) µg/mL.

*V. fischeri* was exposed to 0.1, 1, 10, 100 and 1000 µg/mL of HQ and the percentage loss of fluorescence emission was measured resulting in a EC<sub>50</sub> of 1.446 (1.155-1.796) µg/mL.

HQ has ecotoxicity effects on both aquatic and terrestrial biomarkers, with the invertebrate *D. magna* being particularly sensitive to it. However, from the point of view of environmental relevance, the doses at which HQ exhibits toxicity on these biomarkers are unlikely to be reached in both aquatic and terrestrial environments.

#### 4.01.P-Mo274 Sediment-Water Chironomid Toxicity Test – Experiences Using a Flow-Through Device

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For Environmental Risk Assessments in the frame of several legislations, toxicity tests with sediment dwellers have to be conducted. Internationally accepted OECD guidelines exist for *Lumbriculus* (OECD 225) and *Chironomus* (OECD 218/219). All test designs represent sediment-water compartments. Even when the standard test design is static without water renewal, semi-static or flow-through systems are accepted in exceptional cases (e.g., if water quality becomes inappropriate for the chironomids). Such exceptional cases could also be unstable substances, or precipitating metal salts in spiked water test designs. Despite of most other aquatic or sediment dwelling standard test organisms applied in ecotoxicity tests, chironomids show a holometabolous life cycle starting from a larvae living in and on the sediment surface and ending as a midge, emerging from a pupae at the water surface. Test vessels in chronic tests looking for emergence rate, therefore have to be sealed with emergence traps. This, and the circumstance that the chironomids are inserted as first instar larvae into the overlying water makes the application of a flow-through test system challenging. Additionally, food availability – in most cases a fish food suspension applied via overlying water – could be limited depending on the water exchange rate, affecting the survival rate.

The aim was to perform valid 28-day Sediment-Water Chironomid tests according to OECD 218/219 in a flow-through test system. An adapted flow-through test system developed for *Daphnia* Reproduction Tests was used. First results obtained that the test system ensure remaining of all larvae and pupae in the vessels but problems can arise regarding survival rate when frequently applying fish food suspension as food source. Results for test designs applying more frequently fish food suspension or plant material incorporated into the sediment before test start are currently running and tests comparing the influence of static test design versus flow-through test design on effect concentrations are outstanding.

#### 4.01.P-Mo275 Sediment-Water Hyalella Reproduction Test: Challenges Using Plant Material as Food Source

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For Environmental Risk Assessments in the frame of several legislations, toxicity tests with sediment dwellers have to be conducted. Some legislations offer the chance to reduce an assessment factor when results for three different species are provided. Internationally accepted guidelines exist for *Lumbriculus* (OECD 225), *Chironomus* (OECD 218/219) and *Hyalella* (ASTM E1706). All test designs represent sediment-water compartments. However, according to the OECD and ASTM guidelines, there are two serious differences regarding the feeding regimes for strongly adsorbing substances.

According to the OECD guidelines, ingestion of contaminated food may be a significant exposure route and therefore, the use of food added to the sediment before application of the test substance may be considered. In such a case, finely ground plant material added to the formulated sediment before test substance application must be used instead of frequently applied fish-food suspension via the overlying water. According to the ASTM E1706 guideline, such a feeding regime is not considered.

Independent from the test substance properties, always food suspensions have to be applied frequently via the overlying water. This may be one reasons why the OECD currently develops an own sediment-water *Hyalella* guideline considering different feeding regimes. An international ring test is announced for 2023 and 2024. However, one problem regarding a change in food source and quality could be a reduced survival rate, growth and maybe a total lack of reproduction within the test duration of six weeks. So far, no guideline, ring test or research project published regarding adverse effects of chemicals on *Hyalella* development and reproduction in a sediment-water test system used plant material as the only food source.

In this project we aim to perform valid modified sediment-water *Hyallela azteca* reproduction tests following ASTM E1706 in a flow-through test system using finely ground plant material as the only food source. First results obtained that problems can arise regarding i) fungal growth on the sediment surface and ii) surpassing the threshold value for survival rate. Results for effects on reproduction are outstanding and further tests regarding the origin for the problems (composition of artificial sediment, overlying water quality, food source (species used for plant material), temperature, static/flow-through test system) are currently running.

#### 4.01.P-Mo276 Modified Collembola Test for Granular Formulations

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Plant protection products (PPPs) are currently an essential part of modern agriculture, providing tools for pest and disease control while improving crop yield. Soil organisms are one of the most diverse and abundant communities in agricultural fields and surrounding areas, being non-target organisms of concern regarding potential exposure to PPPs and its consequences. The risk assessment scheme for PPPs for soil macro- and meso-fauna is currently based on Tier 1 laboratory tests with standard species representative of key organism groups (including the *Folsomia candida* reproduction test) and, when an unacceptable risk is found, higher tier tests must be conducted based on field studies. However, improving realism of exposure conditions in Tier 1 tests may provide additional information possibly making higher tier testing unnecessary in the environmental risk assessment of certain PPPs.

To provide an additional option for laboratory testing under more realistic exposure conditions, a methodology was developed for testing the toxicity of granular formulations to *Folsomia candida*. To better mimic field exposure conditions, the method departs from the standard laboratory test methodology adopted in collembolan reproduction tests by using modified test units measuring 17 cm width x 140 cm length x 15 cm height, and an organism density of 0.33 collembolans/cm<sup>2</sup>. The method simulates the field exposure by applying the granules in rows and following application depths mimicking the actual agricultural practice. This strategy allows to evaluate the effect of a granulated pesticide in the density, distribution and reproduction of collembolans in the soil after 28 days of exposure. The developed method and some results on population densities and data of a positive control with boric acid will be presented.

The possibility of manipulating different factors like row width application, inter-row spacing, application depth, population density and different modes of sampling, is one of the advantages the present approach. Furthermore, the possibility of simulating artificial rain or even conducting the entire study under natural conditions may also improve the realism of this approach when compared to laboratory Tier 1 tests. This study design can be easily adapted to not only other Collembola species but also other soil organisms, like earthworms.

#### **4.01.P-Mo277 Malformations in Tadpole Tails, ‘Kinked-Tailed’**

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In studies performed with the African clawed frog (*Xenopus laevis*), a high percentage of the tadpoles (even controls) may present curved “kinked” tails. This condition is occasionally referred as to scoliosis because of the sideways curvature. But scoliosis is associated with a spine, whereas tadpoles carry a fibrous elastic cord, occasionally with notochord cells in the tail. Basically, the kink tail is related to metamorphosis. Tadpoles do not lose their tails, they absorb them. Kink tails are associated with focal apoptosis or even segmental necrosis. Several traumatic conditions, water temperature, UVB-radiation or genetical background may influence the incidence. The presentation summarizes the facts. Other tail and limb findings are discussed and their pathogenesis in relation to endocrine disorders is shown.

#### **4.01.P-Mo278 Mefenpyr Diethyl- an Emerging Safener and its Co-Herbicide, Fenoxaprop-P-ethyl, How Safe?**

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Mefenpyr diethyl, a safener used with active ingredients of Fenoxaprop-P-ethyl herbicide in its formulation and considered inert, has been found in surface water. The potential threats that this safener could cause to non-target aquatic organisms are not yet known, and therefore, the need to study the potential toxicity of these compounds on various endpoints in zebrafish embryos. Acute and chronic studies were conducted on this model organism, with the acute studies targeting mortality and hatchability in zebrafish embryos. The chronic study targeted growth and survival in zebrafish larvae. All methods follow the OECD regulations with slight modification in some instances. Results for MEF alone from the acute studies showed that zebrafish treated with the lower concentrations had no lethal effect, and Zebrafish embryos hatched within the third and fourth day. However, the organisms treated with higher concentrations died in the yolk sac. Results from the mixture of safener and herbicide showed that some percentage of protection was conferred on the organism by MEF against its co-herbicide, FEN, but there were various deformities. These results support the notion that exposure determines toxicity and that the not so studied emerging safeners can cause varying effects from toxicity to lethality and should be monitored and regulated. To understand how these compounds work, we are conducting experiments to understand their mode of action in these organisms, and the results will be presented.

#### **4.01.P-Mo279 Central Composite Rotatable Design Applicability in Ecotoxicological Studies: A Case Study with Aquaculture Drugs and Organic Matter Enrichment Effects on Sea Urchins**

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In Canada, the salmon aquaculture industry is a major economic activity. For fish health control and management, the use of ten drugs and two pesticides in 75% of the facilities between 2016 and 2018 has been reported by science advice documents of Fisheries and Oceans Canada. In-feed products like Emamectin benzoate (EMB), and Oxytetracycline (OTC) enter the marine environment accumulating in sediments, potentially remaining bioavailable to non-target organisms from months to years. Organic matter (OM) content in sediment may affect compounds’ toxicity, bioavailability, and degradation. For example, the degradation product of EMB (Desmethy-EMB) has been detected in aquaculture sites, with one study reporting more toxicity than the parent compound. Despite the regular usage of chemicals in Canadian aquaculture, there are still knowledge gaps regarding environmental fate and effects of mixtures of contaminants on non-target organisms.

In this study, we applied a Central Composite Rotatable Design (CCRD), in long-term exposure of the adult green sea urchin (*Strongylocentrotus droebachiensis*). This species was selected as it plays key ecological and economical roles in coastal ecosystems, is a sensitive organism, and is widely used in ecotoxicological studies.

The sediment concentration ranges of EMB and OTC used in the study were selected based on measurements from more than 400 stations surrounding aquaculture sites across Canada. OM content varies greatly across sites as a result of fish waste and natural geographic conditions. For the CCRD we employed five levels of the three factors: combining environmentally relevant scenarios of EMB (from 0 to 34 ng/g), OTC (0-340 ng/g), and OM (0-10%), for a total of 36 experimental units. The most environmentally common conditions were addressed and included in the design.

We assessed the effect of contaminant mixtures on adult sea urchins' behavior, immunology, physiology, and reproduction, as well as tracked the formation and fate of Desmethyl-EMB under different OTC and OM% conditions.

The data generated in this project will provide an understanding of hazards posed by environmentally relevant concentrations of these compounds and will serve to document potential synergistic/antagonistic effects when establishing environmental quality standards or monitoring thresholds.

#### **4.01.P-Mo280 Proposal for Critical Appraisal Tools for the Evaluation of Ecotoxicology Studies**

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According to the European Regulation (EC) No 1107/2009, pesticide active substances to be approved shall not pose unacceptable effects for human health, animal health and the environment. For this purpose, applicants must submit a dossier, containing a data package including ecotoxicity studies in line with the EU data requirements. The evaluation of such studies, particularly when a standard test guideline is not available, may be challenging and result in inconsistent outcomes.

The scope of the work was to develop Critical Appraisal Tools (CATs) to support the evaluation of the following seven types of non-standard higher tier ecotoxicity studies submitted with these dossiers. Aquatic organisms: Modified exposure studies (tier 2), Mesocosm studies (tier 3). Bees: Honeybee brood test (feeding and tent test version). Non-target arthropods other than bees: Extended laboratory studies, Aged residue studies, Field studies. Residue decline studies and related kinetics (currently relevant for birds and mammals risk assessment): Field studies.

CATs provide a structured and transparent approach to assess the internal and external validity (i.e., reliability and relevance) of individual studies. The work included a systematic literature review of existing CATs and study evaluation methods in ecotoxicology and environmental risk assessment. Based on this review, it was decided to base the CATs to be developed on the CRED approach (Criteria for Reporting and Evaluating Ecotoxicity Data) for evaluating reliability and relevance of studies. The CATs consist of MS-Excel spreadsheets with criteria and scoring tables, accompanied by handbooks with detailed instructions for appraising the individual criteria. A method for evaluating the overall validity of these ecotoxicity studies, based on both a (semi-) quantitative scoring system and expert judgement, is also proposed.

The adoption of common appraisal tools will enhance the harmonisation and transparency of the study evaluations performed by the experts in the area of ecotoxicology and it is expected that it will increase the trust of the scientific assessments conducted by National competent authorities of member states of the European Union and the European Food Safety Authority (EFSA).

#### **4.01.P-Mo281 The Importance of Confidence Limits for Ecotoxicological Endpoints**

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In Europe, Tier I ecotoxicity tests using standard species are a regulatory risk assessment requirement for all chemicals prior to commercial use. The most common statistical endpoints used in acute and chronic ecotoxicological studies are Effect Concentration (EC<sub>x</sub>) values. These describe the concentration at which 'x' percent of the test organisms are affected, for any given parameter. For example, an EC<sub>50</sub> value for mortality would be the calculated concentration where 50% of all test organisms had died.

In recent years, there has been a requirement for the provision of both EC<sub>10</sub> and EC<sub>20</sub> values in addition to the traditional EC<sub>50</sub> values. Therefore, older study data is routinely re-analysed to generate these values to be submitted to the regulatory authorities for current environmental risk assessments.

These re-analyses should also include an assessment of the reliability of each endpoint. This is usually carried out by investigating the corresponding confidence limits. Although confidence limits are a key indicator of the reliability of the data, they can sometimes be overlooked, or their importance misunderstood.

We provide an overview on the current guidance for calculating EC<sub>x</sub> values, and their confidence limits, to provide an insight on how confidence limits can be used to identify a reliable EC<sub>x</sub> endpoint. We will also highlight the standard species accepted endpoints detailed in the OECD test guidelines.

#### **4.01.P-Mo282 The Species Sensitivity Distribution Approach as a Tool for Deriving Safe Threshold Values for Data-Rich Endocrine-Active Substances in the Regulatory Practice: Case Study on Bisphenol A**

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Toxicological thresholds exist for endocrine-active chemicals that are consistent with the fundamentals of ligand-receptor interaction, such as affinity, efficacy, potency, and mass action. For Bisphenol A (BPA), safe thresholds have been derived from species-specific toxicity data representing phylogenetically diverse organisms that cover all trophic levels; the available aquatic toxicity data for BPA allow for the calculation of effects concentrations that are based on exposure-response relationships. We used Species Sensitivity Distribution (SSD) approaches to derive Predicted No Effect Concentrations (PNECs) for BPA using a chronic freshwater toxicity dataset including ED-relevant endpoints that met well-defined criteria for data relevance, reliability and adequacy (e.g., Klimisch scores of 1 or 2). Where appropriate, EC<sub>10</sub> instead of no observed effect concentration (NOEC) values were used as recommended by OECD (2006) and ECHA (2008). We developed both non-parametric and parametric SSDs by fitting the chronic toxicity data to different distributions using regulatory accepted tools, such as the ETx-, MosaicSSD- and the SSDTool-software, and a fitting tool designed by ARCHE. When minimizing the statistical uncertainties around the 5<sup>th</sup> percentile estimate (HC<sub>5-50%</sub>) and considering the goodness-of-fit statistics as recommended by ECHA guidelines, the Weibull distribution was the best fitting distribution for the chronic freshwater toxicity dataset and resulted in an HC<sub>5-50%</sub> of 46.8 µg BPA/L. The normal distribution from the more commonly used ETx tool also provided a well-fit SSD with an HC<sub>5-50%</sub> of 45.2 µg BPA/L. Comparing these HC<sub>5-50%</sub> values with the individual NOEC/EC<sub>10</sub> values from our robust toxicity dataset of 18 species from 15 taxonomic groups results in only one NOEC (25 µg BPA/L) from a study with the mollusk *Marisa cornuarietis* that is below the HC<sub>5-50%</sub> values. However, applying an assessment factor (AF) of 2, which is recommended based on the available information to derive the aquatic PNEC, results in a freshwater PNEC for BPA of 23.4 µg/L using the Weibull distribution or 22.6 µg/L using the ETx tool. Therefore, these PNECs are protective for all species included in this robust chronic freshwater dataset. Our approach demonstrates that statistical extrapolations like SSDs can be used as a tool in the regulatory practice for deriving safe threshold values for data-rich, potentially endocrine-active substances.

#### 4.01.P-Mo283 Assessing Acute Ecological Risks of Selenium to Freshwater Organisms by Species Sensitivity Distributions

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Selenium (Se) pollution is a worldwide phenomenon and is associated with a broad spectrum of human activities, ranging from agriculture to high technology industry processes. The growing interest in selenium toxicity is attributed to the thin line between nutritional value and toxic concentrations. Taiwan's selenium effluent criterion concentration is set at 0.5 mg/L, but this value is higher than the most recommended threshold water quality criterion for the protection of aquatic life of 5 µg/L from conferences. Furthermore, Se emissions are unavoidable in industrialized Taiwan, therefore, we are very interested in protecting the narrow threshold of Taiwan's aquatic environment. In this study, based on species sensitivity distributions (SSD) calculated the acute concentration (HC<sub>5</sub>) to 5% of species from exposure to selenium. The aquatic species used by SSD are mainly the toxicity test species announced by Taiwan, including algae, invertebrates and vertebrates. The results of this study are helpful in obtaining the PNEC (prediction of no-effect concentrations) and HC<sub>5</sub> values of selenium in the water environment, which are rarely discussed but are very important for understanding the environmental management limits of pollutants. The sensitivity of the biota exposed to selenium is, from low to high: *Pseudorasbora parva* > *Cyprinus carpio* > *Chlorella vulgaris* > *Chironomus riparius* > *Neocaridina denticulata* > *Pseudokirchneriella subcapitata* > *Daphnia magna* > *Hyaella azteca*, showing that vertebrates and algae are less affected. In this study, the HC<sub>5</sub> of selenium was 0.311 mg/L and the PNEC value was 0.0622 mg/L. In comparison to the HC<sub>5</sub> of selenium was decreased to 0.114 mg/L and the PNEC value was 0.0228 mg/L. The number of species and the composition of the species greatly influenced the estimation of HC<sub>5</sub>. Ecological risk assessment usually uses the risk quotient (RQ) method, which is the ratio of a predicted environmental concentration (PEC) to a non-effect concentration PNEC, so the larger the PNEC value, the lower the risk. Our research revealed that the risk quotients calculated using laboratory exposure for Se were 0.0305-0.0864, respectively, indicating very low ecological risks. not selected from the database. We believed that the description of local ecological risks is more appropriate than that revealed in database.

#### 4.01.P-Mo284 Development of a chronic OTNE Species Sensitivity Distribution

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Octahydro-tetramethyl-naphthalenyl-ethanone (OTNE) is high volume fragrance ingredient found in cleaning and personal care products, among other applications. A chronic Species Sensitivity Distribution (SSD) was generated to reduce the uncertainty in the OTNE hazard assessment. Species were selected to adhere to SSD criterion for taxonomic diversity. A total of 11 chronic ecotoxicity studies were completed including 3 species of algae (including green, blue-green, and diatom species), 1 aquatic macrophyte, 5 invertebrates (including an amphipod and insect), and 2 fish. The OTNE chronic data were fitted to a log-logistical function from which the HC<sub>5</sub> (SSD<sub>0.05</sub>) and 95% confidence intervals were calculated. The quality of the dataset and the sensitivity and stability of the SSD were validated using recommended criteria and conventional statistical procedures. These included “leave-one-out” and “add-one-in” statistical simulations using hypothetical data. These evaluations demonstrated that the chronic toxicity data were highly ordered, and strongly adhered to statistical assumptions.

#### 4.01.P-Mo285 Towards the Development of Fragrance Specific Ecological Threshold of Toxicological Concern (ecoTTC)

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Threshold for Toxicological Concern, or TTC, is a well-established and regulatory accepted concept in human safety to rapidly screen and assess data poor compounds and mixtures. TTCs are used to establish a de minimis exposure concentration, below which negligible risk is expected. The use of TTCs has been accepted in regulatory safety frameworks for human safety endpoints

and is leveraged by RIFM (Research Institute for Fragrance Materials) to screen fragrance materials in human health safety assessments (e.g., skin endpoints). Efforts are underway to develop an analogous ecological TTC (ecoTTC) approach using distributions of Predicted No Effect Concentration (PNEC) values. The objective of this project is to examine the applicability of the ecoTTC approach for fragrance specific materials. The EnviroTox database was specifically built to support the development and creation of ecoTTCs. This database contains over 4200+ chemicals and over 80,000 aquatic toxicity results. All information was collected from publicly available sources and has not been independently verified for quality. The RIFM proprietary environmental hazard database was explored to further augment the chemical domain of EnviroTox for chemical domains relevant for fragrance ingredients. A fragrance ingredient database was constructed using the robust, high quality ecotoxicity data from the proprietary RIFM database. Unlike the EnviroTox database, all experimental results from the RIFM database were manually reviewed and curated. Studies were assessed for accuracy, data quality, and validity of test methodology. After manual review, 421 acute invert, algae, and fish studies were identified and 48 chronic invert, and fish studies were identified as relevant for ecoTTC development. In effort to create tailored fragrance specific ecoTTC, chemical-class and/or mode of action-specific ecoTTCs will be explored. It is anticipated that the fragrance-specific ecoTTC will update RIFM environmental risk assessment framework to minimize unnecessary animal testing without compromising safety.

#### **4.01.P-Mo286 Data-driven Decision Making Using Advanced High-throughput Environmental Risk Assessment of Fragrance Materials**

*Aurelia Lapczynski<sup>1</sup>, Heather Summers<sup>2</sup> and Christopher Stevens<sup>2</sup>, (1)Research Institute for Fragrance Materials, USA, (2)Integral Consulting*

For the last 20 years, the Research Institute for Fragrance Materials (RIFM) has conducted environmental risk screening and assessments to evaluate the environmental safety of more than 3,000 fragrance materials manufactured and used by its members. Though the RIFM Environmental Framework (Salvito et al. 2002. Environ. Toxicol. Chem., 21: 1301-1308) is an effective tool, in light of rapid growth in global use of fragrance materials, RIFM has updated the framework to expand its geographic scope and to incorporate recent advances in environmental exposure science and ecological hazard characterization. In this presentation we describe the updated Framework, detailing its expanded geographies, real-time access to the most current population and hydrological data, estimated environmental exposure using waste water treatment plant simulation models and biodegradation, material categorization based on mode of action (MoA), application of an MoA-based ecological threshold of concern, and streamlined execution. The tool's methods and outcomes are illustrated through a proof-of-concept exercise. The updated Framework is an enhanced risk assessment tool that enables RIFM and users and suppliers of fragrance materials to perform timely assessments of thousands of fragrance materials to maintain a high degree of environmental protection and support science-based decisions related to product formulations.

#### **4.01.P-Mo287 Risk Assessment of Organic Micropollutant Mixtures based on 5-Year Monitoring Data of Public Wastewater Treatment Plants in Flanders, Belgium**

*Warich Leekitratanapisan and Karel De Schamphelaere, Ghent University, GhEnToxLab, Belgium*

Organic micropollutants (OMPs) are widespread in surface water, which raises concerns about potential risks to aquatic wildlife. One of the main sources of OMPs is effluents of public wastewater treatment plants (WWTPs). In Flanders, Belgium, most of the public WWTPs operate using primary and secondary treatment, which can hardly degrade many of these OMPs. Environmental risk assessment (ERA) is an approach to identify and prioritize problematic OMPs and WWTPs to improve water quality management. In this study, we received a 5-year monitoring database of OMPs from the Flemish Environment Agency (VMM) from 2017-2021, which provides information on measured environmental concentrations. The approaches for ERA included PNEC-based regulatory risk quotients (RQ-PNEC) and toxic units (TUs) for three biological quality elements (BQEs), which are algae (TUalg), crustaceans (TUcrust) and fish (TUFish). 104 OMPs have been detected in concentrations ranging from 4.9 ng/L to 360 µg/L. Based on the RQ-PNEC approach, the predicted environmental risks were mainly driven by the anti-inflammatory drug diclofenac, the anticonvulsant carbamazepine, the herbicide diflufenican, and the X-ray contrast agent iopromide. Herbicides (diuron, terbutylazine, metolachlor) dominate mixture toxicity to algae, whereastoxicity to crustaceans mainly originated from contributions of diclofenac and terbutylazine. For fish, the highest TUs are exhibited by the fungicide carbendazim. Pharmaceuticals and pesticides are two major groups of OMPs in these WWTP effluents posing an environmental risk to aquatic organisms. 94%, 53%, 83%, and 11% WWTP effluents were at high risk of mixture effects with the summation of RQs-PNEC, TUalg, TUcrust, and TUFish exceeding 10, respectively. Additionally, the sum of RQs and TUs of three BQEs appeared to remain constant in the majority of the WWTPs during the most recent 5 years of monitoring. Our calculations suggest that Flanders'public WWTPs need tertiary treatment steps to remove OMPs. Ozonation could be one technology to be implemented to reduce residual toxicity and risks posed by WWTP effluent.

#### **4.01.P-Mo288 Evaluation of the Water Quality of the Cachoeira River, Sc, Brazil**

*Carlos H.L. Soares<sup>1</sup>, Ivana Eunice Baptista<sup>2</sup> and Grasiane Andrietti<sup>2</sup>, (1)Biochemistry, Universidade Federal de Santa Catarina, Brazil, (2)Biochemistry, Federal University of Santa Catarina, Brazil*

The Cachoeira River is the main water resource that crosses a central area of Joinville. In addition to its historical importance in the colonization of the city, this watercourse is responsible for diluting and transporting all kinds of waste. About 50% of the municipality's population resides and, at the same time, promotes your activities at the Cachoeira watershed. Joinville, is characterized by being the main industrial hub in the state, leading the GDP of Santa Catarina. Among the main activities are the textile, metal-mechanical, machinery and equipment, electrical material, auto parts, plastic and furniture industries. The history of surface water degradation at the Cachoeira by industrial and domestic effluents, gives conditions of low quality to this basin. The

objective of this research was to characterize chronic and acute ecotoxicity, using bioindicators such as *Daphnia magna*, *Danio rerio*, *Desmodesmus subspicatus*, physical chemical analysis, quantification of heavy metals and arsenic in the Cachoeira's river. The results obtained indicate that there is a high load of organic contamination by domestic effluents, as well as the introduction of heavy metals, pharmaceutical drugs, illicit drugs and metabolites in the water of the Cachoeira River. The results of the ecotoxicological evaluation showed lethal toxic effects for *Danio rerio*, with the coagulation of eggs, for *Daphnia magna* with the reduction of neonates and decreased longevity, and for *Desmodesmus subspicatus* it was found a tendency to reduce the growth of algae in relation to the control. Therefore, the Cachoeira river does not meet the criteria considered by the current legislation, in several points and parameters.

#### **4.01.P-Mo289 Site-Specific Soil Ecological Risk Assessment on Metal Contaminated Site Based on TRIAD Approach** **Dokyung Kim, Tae-Yang Lee, Haemi Kim, Sun-Hwa Nam, Jin Il Kwak, Lia Kim, Sang A Kim, An Sanghee and Youn-Joo An,** *Konkuk University, Korea*

A TRIAD approach was officially released by International Standard Organization in 2017 as a tool for site-specific soil ecological risk assessment (SERA). The TRIAD approach quantifies the ecological risk of complex contaminants in contaminated site by considering ecotoxicological and ecological factors along with chemical factors. Finally, based on the three line-of-evidences (LoEs), the integrated risk (IR) is calculated as a number between 0 and 1. This study tried to apply the TRIAD approach to metal contaminated site in Korea by estimating the chemical-LoE (Chem-LoE), ecotoxicological-LoE (etox-LoE), and ecological-LoE (eco-LoE). The IR for five cells in site including the reference cell (R) was calculated. The IR of each cell was identified as 0.701, 0.758, 0.840, and 0.429, respectively, confirming a worrisome risk in more than three cells. Among various items used in this study, the extractable concentrations, collembola bioassay, and vegetation survey were found as the best represent item the IR value in each LoE. This study evaluated the applicability of the TRIAD approach via various bioassays and presented reasonable items. Our results can be helpful to select cost-effective bioassay in site-specific SERA based on TRIAD approach.

#### **4.01.P-Mo290 Approach for Environmental Hazard Assessment through Zebrafish Cell Line and Biomaterials** *Indong Jun<sup>1</sup>, Juyong Yoon<sup>2</sup> and Chang Seon Ryu<sup>2</sup>, (1)KIST Europe Forschungsgesellschaft, Germany, (2)KIST Europe, Germany* Numerous methods are being introduced for three-dimensional (3D) cell culture to reduce unnecessary animal experiments. In this study, we present the unique 3D culture platform that featured bioinspired fragments of electrospun nanofibers and aquatic cell lines to compensate for the shortcoming of the current cell spheroid generation technique. Here, it is shown how bioinspired fragments of electrospun nanofibers in 3D zebrafish liver cell culture promote improved functions related to liver and reproduction through spheroid-based in vitro assays, such as whole transcriptome sequencing and reproductive toxicity testing. In addition, it has been demonstrated that the optimized properties of the bioinspired fragments of electrospun nanofiber used in 3D spheroid culture exhibit reproductive toxicity results similar to those of Fish Embryo Acute Toxicity (FET, OECD TG 236) for environmental endocrine-disrupting chemicals exposures (17 $\beta$ -Estradiol (E2), 4-hydroxytamoxifen (4-HT), and bisphenol compounds (BPA and BPFL)). As a closer analog of in vivo conditions responded during exposure to potentially hazardous chemicals, the straightforward culture model introduced in this study shows promise as an alternative tool that can be used to further our understanding of the eco-environmental assessment model. More importantly, this finding can support ongoing development and refinement of the in vitro 3D culture system to accomplish the 3Rs principles (Replacement, Reduction, and Refinement) for alternatives to animal testing.

#### **4.01.P-Mo291 Burden of Disease Assessment for Non-ferrous Metal Industry Air Emissions at a Local Scale** **Joonas Koivisto<sup>1</sup>, Lorenzo Marotti<sup>2</sup>, Violaine Verougstraete<sup>2</sup> and Patrick Van Sprang<sup>1</sup>, (1)ARCHE Consulting, Belgium, (2)Eurometaux, Belgium**

A source/sector specific health impact assessment allows to identify cost-efficient mitigation methods to reduce the air pollution health burden. The source/sector health loss can be quantified by using the Burden of Disease (BoD) concept. The BoD assessment is performed by calculating Disability Adjusted Life Years (DALYs), which combines years of life lost due to premature mortality (YLLs) and years of healthy life lost due to disability (YLDs). BoD assessment is used to estimate the benefits of target emission objectives compared to the currently prevailing situation [1,2]. However, the European Commission revision proposal of the ambient air quality directives lacks examining the relation between emission source/sector and health impact. In this study, the BoD concept was applied to quantify health impacts of the non-ferrous metal (NFM) industry air emissions.

The NFM factory emissions were collected for years 2019 and 2020 from the European Pollutant Release and Transfer Register. An aggregated emission evaluation was performed by using emission weighted stack properties. Gaussian plume dispersion modelling was performed for an area of 1,600 km<sup>2</sup> [3]. The population was set at 800,000 (500 persons/km<sup>2</sup>). Exposure response functions considered: Particulate Matter (PM), SO<sub>2</sub>, NO<sub>x</sub>, CO, Ni, Co, Sb, Cr, Cd, As, and Pb, C<sub>6</sub>H<sub>6</sub>, BaP, dioxins, naphthalene, PCBs, tetrachloroethylene, trichloroethylene and dichloromethane.

Cumulative emissions accounted to 0.51 Mt and 0.55 Mt for the years 2019 and 2020, respectively. The emissions consisted of 90% CO, 6.8% NO<sub>x</sub>, 2.1% SO<sub>x</sub>, 1.1% non-methane volatile organic compounds and 0.5% other pollutants. The calculated average annual ground level concentration was 72.2  $\mu$ g/m<sup>3</sup> at the model area. Calculated DALYs were 1,430 and 1,446 DALYs for years 2019 and 2020, respectively. 96% of the overall DALY burden was covered by NO<sub>x</sub>, PM<sub>2.5</sub>, and PM<sub>2.5-10</sub>. Metals contribution to DALYs was below 0.015% in total. The NFM-industry disease burden including YLD and YLL is 0.04% of YLLs by PM<sub>2.5</sub> pollution in EU-27 in 2019 (4,097,900 YLLs as estimated by the European Environmental Agency [EEA]).

This study shows that the NFM-industry potential to reduce air pollution human health effects is limited compared to other emission categories. A similar impact assessment should be performed for the key emission categories as specified by EEA to identify cost-efficient mitigation methods and to provide evidence for impactful regulatory decision making.

#### **4.01.P-Mo292 Formation of Disinfection By-products (DBPs) in Laboratory Disinfection Simulations Under Different Experimental Conditions**

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Disinfectants are widely used biocidal products with broad scopes of applications including such different fields as (drinking) water, health care, food processing or construction industry. Among the active ingredients used, highly reactive substances like chlorine, peroxides or ozone are common. During their application, numerous disinfection by-products (DBPs) are formed, especially if organic matter is present. The intended use determines possible releases of the formed DBPs into the environment. The authorisation of biocidal products is regulated by the EU Biocides Regulation 528/2012 which also requires the assessment of possible DBPs. The existing "Guidance on Disinfection By-Products" is limited to halogen-containing biocidal active substances and selected product types and includes only general scientific strategies for the risk assessment of DBPs. On this basis a harmonized environmental risk assessment (ERA) of biocidal products and their DBPs within the EU is questionable.

To fill this regulatory gap and develop a feasible regulatory toolkit, the German Environment Agency (Umweltbundesamt, UBA) launched a research project for the consideration of DBPs within the environmental risk assessment of biocidal products (FKZ 3718 65 403 0).

An important part of the project were laboratory simulations of disinfection applications including the analysis for a selection of approx. 60 DBPs. The disinfection simulations were performed using two matrix compositions one focusing on disinfection uses in swimming pools and one containing organic matter that can be generally expected for disinfection uses across the regarded product types (PTs) 1-5, 11 and 12. Simulations were performed for uses in aqueous solutions and on hard surfaces. An elaborate dataset was generated by application of four different biocidal active substances (a.s.) and the variation of different experimental parameters. Significant differences with respect to DBP formation were observed not only for the different biocidal a.s. but also for the two different matrices and the uses in solution and on surface. Key results of the laboratory simulations which give some implications for simulations testing within the ERA of DBPs, will be presented.

#### **4.01.P-Mo293 Development of Simultaneous Determination Method for Urinary Metabolites of 10 Volatile Organic Compounds and Pyrethroid Insecticide**

**Sunhey Jung<sup>1</sup>, Na-Youn Park<sup>1</sup> and Younglim Kho<sup>2</sup>,** (1)Eulji University, Korea, (2)Health, Environment & Safety, Eulji University, Korea

Volatile organic compounds (VOCs) are liquid and gaseous organic compounds with low boiling points that easily evaporate into the atmosphere, and are mainly used in food packaging, paint, and chemical synthesis, and are easily exposed to the indoor or ambient environment. Pyrethroid insecticides are made by artificial synthesis and account for most of the insecticides used in households. These chemicals are metabolized in the body and excreted through urine and simultaneous determination method has not yet been studied. This study aimed to establish a simultaneous analysis method for 11 metabolites of VOCs and pyrethroid insecticides in human urine.

The target compounds were 10 metabolites (2-methyl hippuric acid, 3 and 4-methyl hippuric acid, mandelic acid, phenylglyoxylic acid, trans, trans-muconic acid, N-acetyl-S-(benzyl)-L-cysteine, N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine, N-acetyl-S-(2-carbamoyl-ethyl)-L-cysteine, N-acetyl-S-(N-methylcarbamoyl)-L-cysteine) for VOCs and 3-phenoxybenzoic acid for pyrethroid insecticides. Solid phase extraction (SPE) with a cartridge (HLB 96-Well Plate, 30 µm/30mg, Oasis) was used for urine sample preparation. Targeted compounds were separated on a C18 column (ACME, 2.1x100mm, 1.9µm) with mobile phase A (0.1% acetic acid in water) and B (0.1% acetic acid in acetonitrile). Instrumental analysis was performed by UPLC-MS/MS.

As a results method validation, the accuracies of the established analysis method were range from 82.3 to 115.7%, the precisions were less than 20%, and coefficient of determination ( $r^2$ ) was over 0.995 for all 11 types, showing good linearity, which is judged to be suitable for the analysis of metabolites of VOCs and pyrethroid pesticides in urine simultaneously.

#### **4.01.P-Mo294 Urinary Concentration of Phthalates and Alternative Plasticizer Metabolites in 799 Korean People**

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Phthalates are commonly used as plasticizers in various industries because of their excellent properties of softening plastics. Phthalate has been reported to cause endocrine disorders. Therefore, alternative plasticizers with chemical structures similar to phthalates have been developed and used. However, alternative plasticizers have also been reported to cause endocrine disorders. The purpose of this study was to measure the exposure in human samples to various plasticizers, including several alternative plasticizers for which human exposure assessments have rarely been conducted.

We extracted 799 urine samples among standard human urine samples collected by the Ministry of Food and Drug Safety in Korea in 2016. The selected urine samples were prepared by solid phase extraction, and 38 metabolites of phthalates and alternative plasticizers were analyzed using liquid chromatography-tandem mass spectrometry (TQ 5500+, AB SCIEX).

As a result, 20 compounds showed a detection rate over 70% among 38 target compounds (11 metabolites of phthalates and 9 metabolites of alternative plasticizers). Excluding compounds with a detection rate of less than 70%, the geometric mean (GM) ranged from 0.11 ng/ml for OH-MINCH to 21.70 ng/ml for MnBP. When the samples were classified by sex, most of the GM concentrations of metabolites of plasticizers showed higher in male's urine samples ( $n=411$ ) than in female's urine ( $n=388$ ) except MEP, cx-MEPA and 5cx-MEPTP.

Differences in urine concentrations of phthalates and alternative plasticizers by gender are likely due to differences in lifestyle and behavioral factors. We found that the urinary concentrations of metabolites of plasticizers are higher in children than that of adults, and which was consistent with previous studies. In this study, various plasticizer metabolites were measured in urine. This is the first study to analyze various alternative plasticizers in human samples representing Koreans. Among alternative plasticizers, many metabolites such as DEHTP, DEHA and DINCH were detected.

#### **4.01.P-Mo295 Spatial Temporal Analysis and Risk Assessment of Organic Micropollutants in Western Kenya**

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More than three hundred and fifty thousand (350,000) of chemicals are in daily use for improving the quality of life. However, their occurrence as individual chemical or as a cocktail of chemical mixtures in the environment causes pollution and detrimental effects to organisms. Such kind of chemicals include the often-used organic micropollutants like the pharmaceuticals, pesticides, endocrine disrupting compounds (EDCs), non-prescriptive drugs, personal care products, hormones, steroids, disinfection by-products, and flame retardants among others. Their co-occurrence can result in additive, antagonistic, or synergistic ecotoxicological effects. Their toxicity towards organisms is a function of time, exposure concentration, toxicodynamic and toxicokinetic processes. In this study, we conducted a spatial-temporal analysis and risk assessment of organic micropollutants in twenty-five sites within five rivers located in different regions of western Kenya, where we identified and prioritized compounds based on risk assessment approaches (Toxic unit, Hazard Unit and RQ) on standard test organisms (Fish, Crustacea and algae). Our study was conducted for one year and covered four seasons which are experienced in Kenya, (Two rainy and two dry seasons). The two rainy seasons are experienced between March to May and also between October and November while dry seasons are between June and September and December to February. Our results provide for the first time a broader overview on the contamination of water bodies in western Kenya with mixtures of organic contaminants of emerging concern. The results also play a critical role on a bigger project on the linkage between water contamination and the abundance of host snails of the tropical disease schistosomiasis via toxic effects on competitors and predators supporting the insensitive snail.

#### **4.01.V Advances in Environmental Risk Assessment of Chemicals**

##### **4.01.V-01 Are Existing Bioconcentration Models for Earthworms Able to Account for Inter-Species Differences in Pesticide Uptake from Soil?**

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Earthworms are important ecosystem engineers and as such are commonly included in risk assessment of pesticides in terrestrial environments (SANCO, 2002) and of secondary poisoning via the terrestrial food chain (TGD, 2003). If lower-tier risk assessments indicate potential for effects of pesticides on earthworms, field trials are required to assess impacts more realistically. Modelling approaches that predict internal concentrations of compounds in earthworms from bulk soil or soil porewater concentrations could provide a cost- and time-effective alternative to field testing for characterising the risk of pesticides to earthworms and via secondary poisoning. Several equilibrium partitioning (EP) and dynamic mechanistic models have been developed to predict bioconcentration of organic chemicals in earthworms. EP models assume that the main route of exposure for earthworms is uptake from soil porewater through the skin, and that differences in bioconcentration between earthworm species are negligible. To investigate how existing models perform for different earthworm species, we conducted kinetic uptake and elimination experiments for the earthworms *Eisenia fetida*, *Aporrectodea caliginosa* and *Lumbricus terrestris* which have varying body size and lipid content, in five contrasting soils, and for five pesticides (lenacil, flutriafol, dieldrin, hexachlorobenzene and p,p'-DDT). Measured bioconcentration factors (BCF) were comparable across earthworm species (within a factor of 1.8 on average) for hydrophilic pesticides due to the similar water content of earthworm species. For hydrophobic pesticides, BCF varied between species by an average factor of 4.0, but normalisation of BCF values to earthworm lipid content and specific surface area significantly reduced this to a factor of 1.8. The best performing EP model was that of Belfroid *et al.* (1993), but it still provided poor predictions for *L. terrestris* (NSE= -0.38). This model also overestimated internal concentrations of lenacil for all three earthworm species by up to two orders of magnitude. Existing EP models cannot be generalised to predict bioconcentration across earthworm species. Refined EP models are required separately for hydrophilic and hydrophobic pesticides, incorporating additional soil, and earthworm terms in addition to Log K<sub>ow</sub> to account for the influence of these characteristics on bioconcentration. Further model evaluation and refinement for dynamic bioconcentration models are ongoing.

#### **4.02 Antimicrobials in the Environment – A Threat to Environmental and Human Health?**

##### **4.02.T-01 Dawning of a New ERA: Environmental Risk Assessment of Selection for Antimicrobial Resistance**

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Stevenson<sup>7</sup>, Jane Usher<sup>2</sup>, Adilia Warris<sup>2</sup>, Helen Wilkinson<sup>8</sup> and Lihong Zhang<sup>1</sup>, (1)University of Exeter, United Kingdom, (2)University of Exeter, MRC Centre for Medical Mycology, United Kingdom, (3)Environment Agency, (4)Chief Scientist's Group, Environment Agency (England), United Kingdom, (5)Astrazeneca UK, United Kingdom, (6)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom, (7)Medical School, University of Exeter, United Kingdom, (8)Environment Agency (England), United Kingdom

Antibiotics, antifungals, antimicrobials and other compounds have the potential to directly select or indirectly co-select for antimicrobial resistance. These environmental contaminants are released from a variety of sources, resulting in huge concentration gradients of antimicrobials through different environments. However, a growing body of research has demonstrated that very low concentrations of antibiotics can select for antimicrobial resistance. Therefore, environmental pollution may be directly contributing to the rise of the 'silent pandemic' of AMR, one of the greatest threats to human and animal health, the global economy and global food security.

Currently, environmental risk assessments (ERAs) performed for antimicrobials do not utilise data from experiments that determined the concentration at which an antimicrobial selects for resistance. This has been shown to be orders of magnitude lower than inhibitory, or 'toxic' concentrations in some cases. ERAs using data derived from ecotoxicological experiments may therefore not be protective against antimicrobial resistance selection.

This presentation will outline some of the experimental approaches used to date to determine selective concentrations of antibiotics, the most well studied type of antimicrobial in terms of minimal selective concentrations, and the risk these antibiotics pose in different environments. Antifungals remain unstudied in terms of their minimal selective concentrations, despite being a major contributor to the global burden of antimicrobial resistance. Consideration will therefore be given to how the methods used for antibiotics may be transferred (or not) to studying minimal selective concentrations of antifungals. Finally, issues unique to ERA of antimicrobial resistance will be proposed and outlined in a potential framework to streamline adaption of a new ERA for antimicrobials that considers selection for AMR. These recommendations may help stem the tide of antimicrobial resistance by contributing to the development of environmental quality standards for antimicrobials, when supported by sufficient data.

#### **4.02.T-02 First Steps Towards an Environmental Surveillance for Antimicrobial Resistance (AMR)**

**Wiebke Schmidt<sup>1</sup>**, Jonathan Warren<sup>2</sup>, Collette Allen<sup>3</sup>, Purvi Mali<sup>2</sup>, Elizabeth Johnson<sup>3</sup>, Katie Hopkins<sup>3</sup>, Ian Martin<sup>4</sup>, Alison Mather<sup>5</sup>, Caroline Willis<sup>3</sup> and Alwyn Hart<sup>2</sup>, (1)Chief Scientist's Group, Environment Agency (England), United Kingdom, (2)Environment Agency, (3)UK Health Security Agency, United Kingdom, (4)Environment Agency (England), United Kingdom, (5)Quadram Institute

Traditionally, antimicrobials are associated with the clinical use of antibiotics in the protection of human and animal health, but antimicrobials are also used for crop protection. We know that the presence and pathways of antimicrobial substances and resistant organisms are not limited to the intended spaces (e.g., hospital, farmyard) and can enter our environment. For example, antifungals are spread across large, farmed areas at effect concentrations or antibiotics excreted by patients enter into the sewage system and ultimately into water environment. Thus, we need to understand the role that the environment plays in the development, maintenance and transport of antimicrobial resistance (AMR) and the impact of AMR on environmental ecosystems.

Globally, organisations including the World Health Organisation, United Nations Environment Programme and the UK Government have identified and called for a need for surveillance of antimicrobial resistance in the environment to sit alongside the surveillance carried out in clinical and veterinary settings. However, having recognised the need for environmental surveillance there are multiple challenges in deciding what this should entail.

The Environment Agency, UK Health Security Agency and Department for Environment, Food and Rural Affairs are piloting how to deliver AMR surveillance in the environment in England, UK. We will present results how we conducted sampling across different environmental matrices, with a focus on three river catchments in England. In addition to presenting results from our river catchment works, we shall showcase results from other areas, such as presenting detected concentrations of clinical antifungals in biosolids in England as well as results from using shellfish as bioindicators for coastal waters. Our pilot work also considers practical considerations, such as site access, resource requirements and usage of results that need to be taken into account for the development of a future environmental AMR surveillance programme.

#### **4.02.T-03 Terrestrial Risk of Antibiotic Resistance in Slurry or Manure Amended Soils**

**Felicity C T Elder<sup>1</sup>**, Alex J O'Neill<sup>2</sup>, Lisa Collins<sup>2</sup> and Laura Carter<sup>3</sup>, (1)Geography, University of Leeds, United Kingdom, (2)University of Leeds, United Kingdom, (3)School of Geography, University of Leeds, United Kingdom

Antibiotic resistance (ABR) is a current and very real global health and economic threat, as antibiotics are fundamental in both healthcare and food production. There is increasing concern that the presence of antibiotics in the environment may select for the evolution and dissemination of antibiotic resistance. Currently there are no regulatory guidelines that take these risks into account, and while there is ongoing work to address this within aquatic environments, terrestrial systems have been somewhat overlooked, mainly due to our focus on wastewater treatment plant effluent as the main source of antibiotics within the environment. There is an increasing push within agriculture to move away from chemical-based fertilisers and towards the use of organic soil amendments such as slurry, manure or sludge, in order to improve soil health. However, these organic soil amendments have been shown to contain antibiotics and other pharmaceuticals alongside antibiotic resistant bacteria posing a potential risk to the environment, livestock and humans through the proliferation and spread of ABR. Using current knowledge on the fate of antibiotics within soil and mathematical models to enable an assessment of risk of ABR, this presentation puts forward a novel framework for assessing the terrestrial risk of antibiotic resistance through the use of farmyard manure as fertilisers, alongside experiment data to show the potential for predicted pore water concentrations to select for oxytetracycline resistance.



#### 4.02.T-04 Presence and Dissemination of Antibiotic Resistance in Agricultural Soil Supplemented with Sewage Sludge

**Marta Jaskulak<sup>1</sup>, Katarzyna Zorena<sup>2</sup>, Saeid Rostami<sup>3</sup>, Damian Lech<sup>2</sup>, Maciej Winczewski<sup>2</sup>, Jakub Krajnik<sup>2</sup> and Franck Vandenbulcke<sup>3</sup>, (1)Czestochowa University of Technology, France, (2)Medical University of Gdansk, Poland, (3)University of Lille, France**

For the last 20 years in EU-15 countries, the sewage sludge produced by wastewater treatment plants (WWTPs) has increased from 6.5 million tDM (dry matter) up to 9.5 million tDM (2000-2020). Applying sewage sludge (SS) to agricultural soil can improve the soil fertility, allows to recycle this biowaste and helps to meet crop nutritional needs. However, SS may contain various unregulated emerging contaminants, as well as mobile genetic elements (MGEs) and antibiotic resistance genes (ARGs), increasing the danger of the spread of antibiotic resistance. We looked at how applying thermally dried anaerobically digested SS affected the physical, chemical, and microbiological characteristics of the soil, and (ii) the relative abundance of 96 ARGs and 10 MGE-genes in the soil. Three factors—the dose of application, the technology of sludge treatment, and the amount of time since the last treatment—were different for each of the SS-amended agricultural fields from which soil samples were collected. ARGs and MGE-genes were both more abundant in soils fertilized with sewage sludge relative to non-amended soils in SS-amended soils, particularly in more recent SS applications and in soils supplemented by SS repeatedly thorough the last 5 years. The relative abundance of ARGs and MGE-genes was positively linked with some physicochemical factors, such as cation exchange capacity, nitrogen and phosphorus content. The primary explanation for the distribution pattern of ARGs and MGE-genes was the application of sewage sludge. We concluded that adding thermally dried anaerobically digested sewage sludge to agricultural soils without any additional treatments can enhance the chance of antibiotic resistance spreading. Much future research needed for the development of innovative treatments and technologies for SS to reduce the risk of further emergence and spread of ARGs and MGE-genes in SS-amended agricultural soils and afterwards, in crops. Until then, SS should be applied in agricultural soils with caution.

#### 4.02.T-05 Assessing the Chemical Complexity of Discharge into the Environment from Antimicrobial Manufacturing Hubs in India

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Our current understanding of the presence of antimicrobial resistance (AMR) drivers in the environment has been driven by a targeted approach to chemical identification, whereby potential drivers of AMR may be missed. To understand the complexity of chemical contaminants released into the environment following pharmaceutical manufacturing activities, we carried out a non-targeted approach using high-resolution mass spectrometry to identify antimicrobials and other chemical contaminants in environmental samples across two sites known as antimicrobial manufacturing hubs in India. Compound discovery software was used for data analysis, where existing libraries (m/z cloud and Norman mass list) were used for compound annotation. Suspect lists were also created from information regarding the known product formulations from antimicrobial industries in the local sampling area. A workflow was used to clean the data and included steps to remove chemicals identified with no name and a delta mass > 5 ppm. Peak area (< 50,000 removed) and peak rating (< 5 removed) were then assessed. In the initial screening, at level 5 confidence, the hit list consisted of 4914 compounds identified in samples of wastewater influent, effluent and river water (upstream and downstream). In total, 458 chemicals were annotated to L3 Schmanski confidence, of which 57 chemicals were confirmed as L2 confidence following a spectral library match with m/z cloud. Of the chemicals annotated as L2, sulfamethazine, methicilin and metampicilin antibiotics were found repeatedly in samples from Bhiwadi, Rajasthan and Dera Bassi, River Ghaggar. In addition, almost all collected samples contained beta blockers, antidepressants and illegal drugs. Chemicals were still detected within the effluent following wastewater treatment suggesting that current treatment options are ineffective and are contributing to the direct release of AMR drivers into the receiving environment. Most importantly, it demands further investigation into the impact of these ubiquitous contaminants on human and environmental health and highlights that environments surrounding manufacturing facilities contain a complex cocktail of chemicals beyond just active antimicrobial ingredients.

#### 4.02.P Antimicrobials in the Environment – A Threat to Environmental and Human Health?

##### 4.02.P-Mo296 Veterinary Antibiotics in the Soil Environment: Earthworm *Dendrobaena veneta* Response to Tetracycline Exposure

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Antibiotics have received growing attention in recent years as emerging aquatic and terrestrial contaminants. Animal manure, slurry or sewage sludge land application are the main routes for veterinary antibiotics to enter the soil environment. Tetracyclines are among the antibiotics most used in veterinary resulting in their wide distribution in the environment. However antibiotic effects on soil dwelling organisms are still poorly understood. Earthworms *Dendrobaena veneta* were exposed to 1-500 mg/kg of tetracycline for 56 days. Mortality, body weight, cocoon production and biochemical responses including the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST) and malondialdehyde (MDA) were examined. Tetracycline reduced *D. veneta* survival, weight growth, and impaired antioxidant enzymes system. Data on *D. veneta* response to tetracycline will be presented.

**4.02.P-Mo297 Effects of Tetracycline on Physiological and Enzymatic Defense Response in Lichen *Evernia prunastri***  
Gintare Sujetoviene<sup>1</sup>, **Diana Miškelytė<sup>1</sup>**, Aušra Dikšaitytė<sup>1</sup>, Irena Januskaitienė<sup>1</sup>, Giedrė Kacienė<sup>1</sup>, Jūratė Zaltauškaitė<sup>2</sup> and Martynas Jasas<sup>1</sup>, (1)Vytautas Magnus University, Lithuania, (2)Lithuanian Energy Institute, Lithuania

Tetracycline antibiotics are one of the main groups of antibiotics widely used in veterinary medicine, human medicine, and agriculture, with significant environmental concerns including ecological risks. The impact of tetracycline on physiological parameters (integrity of cell membranes, membrane lipid peroxidation, chlorophyll content, photosynthetic efficiency) and enzymatic activity of lichens was determined. Lichens *Evernia prunastri* were exposed to different concentrations: 0, 10, 50, 250, 500 µg/L. The lichens were incubating with tetracycline solutions for 2 h (simulating a single pollution episode) maintaining a constant 10/1 w/v ratio. The results of the experiment showed that exposure affected the lichen membrane damage as indicated by increase in conductivity. The potential photosystem II efficiency ( $F_v/F_m$ ) was susceptible to the impact of antibiotic. The results of the study supplemented the knowledge on the effects of pollution on organisms, such as veterinary antibiotics, on lichens and provided a better understanding of the mechanisms of toxicity.

**4.02.P-Mo298 Pre-COVID-19 Occurrence of Antimicrobials, ARB, and ARGs in Hospital Wastewaters and Adjacent Surface Waters in Sri Lanka**

**Keerthi S. Guruge<sup>1</sup>**, Yukino A Tamamura<sup>1</sup>, Prasun Goswami<sup>1</sup>, Rumi Tanoue<sup>2</sup>, K. B. S. N. Jinadasa<sup>3</sup>, Kei Nomiyama<sup>2</sup> and Tatsuya Kunisue<sup>2</sup>, (1)National Institute of Animal Health-NARO, Japan, (2)Ehime University, Japan, (3)University of Peradeniya, Sri Lanka

In this study, the concentrations of 25 antimicrobials, the resistance of *Escherichia coli* (*E. coli*) strains in response to the selection pressure imposed by 15 antimicrobials, and enrichment of 20 antimicrobial resistance genes (ARGs) in *E. coli* isolated from hospital wastewaters and surface waters were investigated during the pre-COVID-19 period, from 2016 to 2018. In hospital wastewaters, clarithromycin (18,800 ng/L) was detected at the highest concentration followed by sulfamethoxazole (15,300 ng/L) and sulfapyridine (14,120 ng/L). The maximum concentrations for personal care products (PCPs) such as triclocarban, triclosan and methyl paraben were detected at 363 ng/L, 77 ng/L and 468 ng/L, respectively, in the hospital wastewaters. Approximately 80% of the *E. coli* isolates were resistant against the tested antimicrobial agents. Approximately 61% of the examined isolates were categorized as multidrug-resistant bacteria. The overall abundance of phenotypes that were resistant toward drugs was in the following order:  $\beta$ -lactams, tetracycline, quinolones, sulfamethoxazole/trimethoprim, aminoglycosides, and chloramphenicol. The data showed that the *E. coli* isolates frequently harbored *bla*TEM, *bla*CTX-M, *tetA*, *qnrS*, and *sul2*. These results indicated that some PCPs were significantly associated with the presence of several resistant phenotypes and resistance genes, implying their role in co-association with multidrug resistance. Statistical analysis also indicated a disparity specific to the site, treatment, and year in the data describing the prevalence of antimicrobial-resistant bacteria (ARB) and ARGs and their release into downstream waters. This valuable information on pre-COVID-19 abundance of antimicrobial, ARB and ARGs can be compared with the With/Post-Covid-19 occurrence of them in Sri Lanka.

**4.02.P-Mo299 Impact of Thermal Hydrolysis on Abundance of Antibiotic Resistance Genes During Anaerobic Digestion of Sewage Sludge**

**Young Mo KIM**, Department of Civil and Environmental Engineering, Hanyang University, Korea

The effects of recuperative thickening (RT) and thermal hydrolysis during anaerobic digestion (AD) on AD performance, microbial communities and reduction of antibiotic resistance genes (ARGs) were investigated. Compared to the results in conventional AD (Phase I), biogas production increased slightly (10%) through RT (Phase II) but increased (63%) via RT with a digestate treatment system (DTS, thermal hydrolysis of thickened digestate) during AD (Phase III). Meanwhile, the three different operating conditions further influenced microbial community structures as well as efficiencies in reducing ARGs: The abundance of hydrolytic bacteria in Phase II and the abundance of acidogenic bacteria in Phase III increased, and 64.3% and 47.4% of the total sum of the relative abundance of ARGs in the substrate were removed in Phases II and III, respectively. Thus, RT-AD with DTS could represent a promising process for improved AD performance, but requires additional refinements to further reduce concentrations of residual ARGs in digestate.

**4.02.P-Mo300 Characterizing Antimicrobial Resistance Across Australia**

Jake O'Brien<sup>1</sup>, Aimee Kaye Murray<sup>2</sup>, **Leah Clarke<sup>1</sup>**, Kevin Thomas<sup>3</sup> and William Gaze<sup>2</sup>, (1)Queensland Alliance for Environmental Health Sciences (QAEHS), University of Queensland, Australia, (2)University of Exeter, United Kingdom, (3)University of Queensland, Australia

Antimicrobial resistance (AMR) looming threat to public health world-wide. Resistant bacteria can be found in clinical settings and throughout the natural environment. When antibiotics and antimicrobial compounds are consumed by humans or animals, they do not completely break down during metabolism and are excreted into the environment - whether that be via wastewater treatment plants, animal manure, aquaculture reservoirs, and water bodies.

The objective is to characterize antibiotic resistance in wastewater using both analytical chemistry and sequencing techniques. To facilitate this, wastewater samples have been collected and preserved from over 100 wastewater treatment plants across Australia since 2016. Importantly, area specific demographics have also been collected for each of the wastewater catchments in order to provide a socioeconomic dimension to the data.

**4.02.P-Mo301 Certifying the Responsible Manufacture of Antibiotics**

**Joan G. Tell<sup>1</sup>** and Daniel Caldwell<sup>2</sup>, (1)MSD, (2)Johnson & Johnson

The AMR Industry Alliance (the Alliance) brings together the leading biotech, diagnostic, generic and research-based

pharmaceutical companies around the shared goal of curbing antimicrobial resistance in the world. Alliance companies are committed to contribute to and measure their efforts in fighting AMR across four key areas: research, appropriate use, access and manufacturing and the environment. In 2018, AMR Alliance generic and research-based pharmaceutical companies agreed on a framework that promotes responsible antibiotic manufacturing and derived Predicted No-Effect Concentrations (PNECs) for antibiotics in surface water that would be protective of ecological receptors and also limit the spread of resistance from manufacturing sites. Since 2018, the Alliance has conducted additional environmental tests on antibiotics, provided guidance to manufacturers on best practices, set default values to use in the absence of data and are furthering the science via targeted research grants. With the help of the British Standards Institute, the Alliance has developed a **standard and certification scheme** that encompasses all the necessary elements of responsible manufacturing of antibiotics in a protective and transparent manner. The certification scheme will provide a level of assurance for the general public, regulators and procurers that the antibiotics they use and purchase are made in a manner that is safe for the environment and minimizes the contribution of manufacturing to the spread of resistance.

#### **4.02.P-Mo302 Detection of Antimicrobials in Common Effluent Treatment Plant Wastewater and Receiving Water Body** **Arhamia Tufail Ahd Ansari, Ayush Ransingh, Pradnya Vernekar and Suparna Mukherji, Environmental Science and Engineering Department, IIT Bombay, India**

In recent times, a large quantity of antibiotics is being administered to humans and animals for treating diseases and infections. This has increased the production of antimicrobials and the generation of antimicrobial-containing effluents. In India, various drug and pharmaceutical industries send their effluent to common effluent treatment plants (CETPs), where wastewater from various types of industries is treated together. In the present study, antimicrobials in CETP influent, effluent, and samples from the receiving water body were analyzed using solid-phase extraction followed by LCMS-MS using concentration factors 100, and 500, respectively. The antibiotics detected in both influent and effluent wastewater samples from the CETP included cefalexin, netilmicin, enrofloxacin, erythromycin, azithromycin, and sulfamethazine. Some of the antibiotics were detected only in the effluent samples. Several of the antibiotics detected in the effluent samples were also found in the water samples from the receiving water body. Along with antibiotics, other antimicrobial compounds, such as antifungals and antivirals, were also detected in the CETP treatment plant and the receiving water body.

#### **4.02.P-Mo303 Investigating the Effects of Aminoglycoside Antibiotic Plant Protection Products on Complex Microbial Communities**

**Laura May Murray<sup>1</sup>, Lihong Zhang<sup>1</sup>, Jason Snape<sup>2</sup>, William Gaze<sup>1</sup> and Aimee Kaye Murray<sup>1</sup>, (1)University of Exeter, United Kingdom, (2)Astrazeneca UK, United Kingdom**

Crop production utilises the application of plant protection products (PPPs), including antibiotics, to crops and soils in many countries. This targets bacterial crop pathogens but results in both target and non-target organisms being exposed to concentrations of antibiotic up to hundreds of mg/L. Bacterial exposure to antibiotic PPPs may result in the development and spread of antibiotic resistance genes (ARGs) between environmentally and clinically relevant bacteria. This project considers how use of antibiotic PPPs contributes to the burden of antibiotic resistance (ABR) via a novel comparison of different antibiotics belonging to the same class, which are crop-specific (kasugamycin) or used both clinically, and as PPPs (streptomycin and gentamycin).

Lowest observed effect concentrations (LOECs) for a reduction in growth of a complex bacterial community were determined for streptomycin (2mg/L), gentamicin (0.25mg/L) and kasugamycin (50mg/L) using over-night growth experiments. LOECs informed the concentration range for seven-day evolution experiments. QPCR was performed on extracted DNA from evolved communities to determine *intI1*/16S rRNA prevalence. Metagenomic sequencing was conducted and analysed to determine changes in community composition (MetaPhlAn2) or relative abundance of ARGs (ARGs-OAP).

Streptomycin and gentamycin significantly increased *intI1* prevalence at concentrations orders of magnitude lower than application concentration. Increases in specific ARG classes (e.g. aminoglycosides & beta-lactams) were also observed. Species richness also decreased with increasing antibiotic concentration. Kasugamycin did not significantly increase *intI1*, nor strongly select for ARG classes even at its application concentration of 100mg/L. Kasugamycin as a crop-specific antibiotic, is less well researched than the others, therefore resistance genes may not have been characterised. We aim to carry out functional metagenomics to identify novel kasugamycin resistance genes.

Results provide information on the effects of different PPPs on microbial communities, contributing to understanding ABR from a “One Health” perspective. Results demonstrate that antibiotics of the same class may have different selective capabilities, necessitating further studies on AMR selection at the level of the individual antibiotic rather than class level.

#### **4.02.P-Mo304 Validation of the SELECT Assay as a Method to Facilitate Risk Assessment for Antimicrobial Resistance** **Alejandra Bouzas Monroy<sup>1</sup>, Ville Friman<sup>2</sup>, John Wilkinson<sup>3</sup> and Alistair B.A. Boxall<sup>4</sup>, (1)Environment, University of York, United Kingdom, (2)Biology, University of York, United Kingdom, (3)Environment and Geography, University of York, United Kingdom, (4)University of York, United Kingdom**

Antimicrobials are widely used to treat humans and animals and during their manufacture, use and disposal it is inevitable that they will be released to the natural environment. Residues of antibiotics have the potential to select for antimicrobial resistance (AMR) in bacteria and contribute to the global AMR crisis. It is therefore critical that concentrations of antimicrobials in the environment are managed but to achieve this it will be necessary to derive safe target concentrations. Recently, the ‘SElection End points in Communities of bacTeria’ (SELECT) method was proposed as a simple approach to derive safe concentrations of antimicrobials in the environment (Murray et al. 2020). However, to date, this approach has not been independently evaluated and

assessed. In this study, we used SELECT, culture-based assays, and qPCR-based assays to determine 'safe' concentrations of 14 antimicrobials (azithromycin, cefotaxime, chloramphenicol, ciprofloxacin, clarithromycin, erythromycin, gentamicin, trimethoprim, metronidazole, sulfamethoxazole, sulfadiazine, lincomycin, tetracycline, and tylosin) in the environment. Bacterial communities from a range of influent samples obtained from plants with a variety of treatment system types and sizes were exposed to a range of concentrations of each antimicrobial using the different assay approaches. Results for the different bacterial communities and the different assay types were then compared to understand the performance of the SELECT assay.

#### 4.02.P-Mo305 Impacts of Human-use Antifungals on Symbiotic Soil Fungi in the Agro-Environment

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To sustainably meet the future food demands of a growing human population, regenerative or circular farming practices must be implemented. Central to these is preservation and exploitation of beneficial soil microbes, such as arbuscular mycorrhizal fungi (AMF), to improve soil structure, increased access to soil nutrients, and enhanced stress protection in crops. As the production of artificial fertilizers rely on finite natural resources, sustainable crop production should aim to maximise the potential benefits of crop-AMF interactions. Irrigation using wastewater and biosolids can also reduce the application of artificial fertilizers, thereby improving sustainability of crop production. However, this increasingly common practice also represents a pathway for man-made chemicals to enter agricultural soils. Emerging pharmaceutical contaminants exist in soils at concentrations ranging from ng/kg – µg/kg and have manifested in sub-lethal effects on plants. Here, we focus on human-use antifungal azoles, bioactive pharmaceuticals that retain their biological potency when present in soil. The inadvertent introduction of human-use antifungal azoles to agricultural soils could negatively impact many aspects of soil health, including abundance, structure, and function of AMF. These near-ubiquitous symbiotic soil fungi are critical components of soil health through their contributions to soil nutrient cycling and plant nutrition, but the effects of antifungal azoles on AMF are unknown. We tracked carbon for nutrient exchange between AMF and two host crops, spring onion and lettuce, in the presence of environmentally relevant concentrations of antifungal azoles. We found that azoles greatly reduced AMF density in the soil and colonization of crop roots. AMF function was also significantly altered, with reduced fungal-acquired phosphorus allocation to host plants and reduced plant carbon allocation to AMF in both species tested when azoles were present. Our results suggest that the presence of human-use antifungal azoles in soils negatively affect the function of AMF with potential subsequent impacts on plant and soil health. While the need for greater sustainability in future farming practices is clear, our research shows there can be significant, albeit unintended, impacts on the soil ecosystem of circular agricultural practices. As such, wastewater treatment processes must be improved to remove the emerging threat of human-use pharmaceuticals.

#### 4.02.P-Mo306 Cell and Tissue Level Effects of Sulfamethazine and Tetracycline in the Earthworm *Eisenia fetida*

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Sulfamethazine and Tetracycline are two common antimicrobial compounds widely used in health services and cattle industry. Therefore, it is not unexpected finding relatively relevant concentrations of both substances in soils due to uncontrolled disposals or other careless management policies. Although broadly used, the information of the effects exerted by these compounds in a soil key species such as earthworms is still scarce. In the present work, earthworms (*Eisenia fetida*) were exposed to three different sulfamethazine and tetracycline concentrations (10, 100 and 1000 mg / Kg soil dw) to determine how these compounds may alter earthworm health status using cell and tissue level biomarkers including enzyme activity measurements, histochemical and histopathological alterations. Preliminary results indicated that after 14 days of exposure even though none of the antimicrobials induced earthworm death or significant weight loss, some cell levels changes were observed. Thus, a trend to increase of riboflavin concentration of the coelomic fluid was observed under the exposure of both compounds even at low concentrations (10 mg / Kg soil dw). On the other hand, also a trend to decrease the height of the epithelium of the digestive tract was observed. This trend was more marked in earthworms exposed to sulfamethazine than in those exposed to tetracycline although in the latter a decrease of digestive tract irregularities and concomitant decrease of soil contact surface was detected. Similarly, the mucous secretion of the earthworm integument presented different response patterns according to the antimicrobial. In the case of the sulfamethazine an increased volume density of mucocytes was measured while no clear trend was observed in tetracycline exposed earthworms. Overall, it can be concluded that some effects were observed at cell and tissue level after exposure to antimicrobials but these changes did not induce more significant alterations in terms of individual mortality or weight loss after 14 days of exposure.

#### 4.02.P-Mo307 Effects of Legacy and Emerging Antimicrobial Compounds to Early Life Stages of Rainbow Trout (*Oncorhynchus mykiss*)

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Products with antimicrobial properties can be released with wastewater effluent and enter freshwater systems, where they might pose a risk to the health of aquatic organisms. While the effects of legacy antimicrobial compounds, such as triclosan (TCS), are well-studied, less is known about the toxicity of emerging alternative antimicrobials. This study aimed to evaluate and compare

the effects of TCS and two commonly used and high-volume antimicrobials, chloroxylenol (PCMX) and methylisothiazolinone (MIT), on early-life stage rainbow trout (*Oncorhynchus mykiss*). Embryos were exposed from hatch until 28 days post-hatch (dph) to TCS, PCMX, and MIT separately at nominal concentrations of 0.38 – 400 µg/L. After 96 h exposure (4 dph), whole embryos were sub-sampled and pooled per concentration to assess transcriptomic responses using a novel standardized reduced transcriptome qPCR array (EcoToxChip) specific to rainbow trout. The remaining individuals were carried through the 28-day exposure, during which mortality along with sublethal developmental responses (e.g., presence of edema and spinal curvature, time to swim-up) were recorded. At study termination, individuals were weighed, length recorded, and samples were preserved for histology of key tissues (liver, gills, intestine). Water was sampled throughout the experiment to confirm exposure concentrations. There was a reduction in survivability and increased incidence of developmental abnormalities (primarily edema and jaw deformity) in fish exposed to TCS and PCMX at the higher exposure concentrations as compared to MIT. Reduced transcriptome profiling using the EcoToxChip is pending, but this broader evaluation of gene expression is anticipated to reveal additional mode(s) of action for these compounds. This research will provide information on the biological effects of antimicrobials on an ecologically relevant fish species and provide data to inform risk assessment of these compounds in Canadian freshwater systems.

#### **4.02.P-Mo308 Potential of the microalgae, *Chlorella sorokiniana*, in the Removal of Nutrients and Antimicrobials in Wastewater Treatment**

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Access to clean water is a global priority as well as a focus of the UN SDGs. An increasing number of frequently detected contaminants in the aquatic environment include pharmaceutical contaminants (antimicrobials), which represents a significant risk to human and environmental health and the spread of antimicrobial resistance.

Microalgae represent a potential new direction in sustainable water treatment with application in the bioeconomy and circular economic systems. These organisms offer the opportunity to provide “bioremediation” as they utilise excess nutrients in wastewater for growth and offer the ability to decompose organic pollutants or accumulate these compounds in their biomass. Algae can remove dissolved nutrients in the form of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and COD. Pollutants removal in this system is facilitated through several mechanisms, such as photodegradation, bioadsorption, and biodegradation.

In this experiment, *C. sorokiniana* (CS) was used to measure the removal of a mixture of 10 antimicrobials, selected based on environmental risk assessment using modelled wastewater concentrations derived from annual mass prescription data available through the NHS in the UK. The selected antimicrobials had a risk quotient > 1 calculated by the ratio between PEC and PNEC. Experimental antimicrobials concentrations were prepared according to the calculated PEC values.

Four different batches of the experiment were designed to examine the growth effect of the algae cells of treatment compared to the control over 19 days. Abiotic controls were conducted to determine the mechanism involved in the elimination of antimicrobials.

The achieved results from the experiment showed that CS was effective in eliminating antimicrobials > 85 % in the  $\beta$ -lactam class. Photodegradation also contributed to the highest removal of tetracycline class at 75 %.

Analysis of the biodegradation mechanism revealed a direct effect on the increased cellular concentration of algae suggesting the utilization of antimicrobials as a carbon source for algal growth and the  $\beta$ -lactam class had the highest removal (83 %). The effect on dissolved nutrients elimination was also studied. The  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and COD demonstrated increased removals over controls at 88, 22, 100, and 10 %, respectively.

The results of the study confirmed the ability of CS to bioadsorb, and potentially biodegrade antimicrobials while also effectively reducing key nutrient loadings including  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and COD.

#### **4.02.P-Mo309 Impacts of Three Antimicrobials on Early-Life Stage Rainbow Trout Gut Microbiome**

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Antimicrobials are contaminants of emerging concern as they are continuously discharged via wastewater effluent in urban environments and their pseudo-persistence may negatively affect organisms in the receiving ecosystem, including fish. In particular, antimicrobials may alter fish gut microbiomes which serve central roles in the overall condition of hosts and therefore could have important implications for the health of host fishes. This study assessed the impacts of three antimicrobials on early-life stage rainbow trout gut microbiome. Embryos were exposed from hatch to 28 days post-hatch to six graded concentrations of triclosan (TCS), methylisothiazolinone (MIT), and chloroxylenol (PCMX). Excised whole gut was collected and 16S rDNA amplicon sequencing of gut microbiome was applied to investigate responses to antimicrobials. Gut microbiome was dominated by phyla Proteobacteria and Bacteroidota and genera *Aeromonas* and *Pseudomonas*. Significant changes in Shannon diversity were observed in TCS-exposed fish, with PCMX-exposed fish indicating a marginal response. Differences in community composition were shown for TCS, with slight changes being observed for MIT. Fourteen genera correlated with nominal concentrations of TCS, while PCMX and MIT each have a single genus significantly correlated. TCS overall had the largest impact on exposed fish gut microbiome, followed by PCMX and MIT. Future steps include assessing the functional response of the gut microbiome to antimicrobials using predictive bioinformatics tools and assessment of antimicrobials on rainbow trout gut cell line (RTgutGC) to assess possible direct impacts of the chemicals on intestinal tissue. Results of this study provide insights into the potential effects of emerging antimicrobials on alevin rainbow trout gut microbiome with implications on physiological health status of the host.

#### 4.02.P-Mo310 Characterisation of Antimicrobial Resistance in Wastewater Treatment Biosolids

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Antimicrobial resistance (AMR) is a global public-health crisis, threatening lives worldwide. AMR poses a difficult challenge since both exposure to antimicrobials and transfer of antimicrobial resistance genes (ARGs) contribute to its spread. Wastewater treatment plants (WWTPs) and their by-products are hotspots of AMR and reservoirs for antimicrobial compounds and ARGs. As WWTPs receive the unmetabolised portion of antimicrobials excreted by a population, the mixture of antimicrobial compounds and subsequent ARGs throughout is complex.

The by-products of wastewater treatment, namely biosolids and effluent are likely to contain the aforementioned complex mixture, and these by-products are often released, or reused within the surrounding environment, serving as a pathway for contaminant exposure. Biosolids, once separated from the liquid stream of wastewater through centrifugation, are usually dried or dewatered before reuse within the agricultural industry as organic fertilisers or soil conditioners.

Previous work has tentatively identified 119 different ARGs and 23 antimicrobial parent compounds from samples taken from Naburn WWTP on a snapshot day in 2020. Methods included screening with SmartChip™ HT-qPCR for ARG identification and high-resolution LC-MS/MS, coupled with a custom workflow developed within Thermo CompoundDiscoverer for chemical analysis. Associations between major antibiotic classes including aminoglycosides, beta-lactams and tetracyclines and their subsequent resistance genes were determined. These results provide new insights into the dynamics of AMR within a critical hotspot in wastewater treatment.

Further study investigating the fate of these ARGs and antimicrobial compounds was conducted over the 28-day air-drying period for biosolids. Samples were collected from 6 different spatial points within the ~200 T biosolid pile twice a week, for four weeks. ARG and chemical analysis provide insight into the dynamics of these pollutants and the ability to evaluate biosolid drying as a process meant to reduce their environmental exposure. Results show that trends in contaminant loading over time are compound and gene specific and enable data to inform pollution management options aimed to reduce the environmental burden of antimicrobials and antimicrobial resistance.

#### 4.02.P-Mo311 Quaternary Ammonium Disinfectants in Livestock Farms and Human Wastewater Treatment Systems – a Driver of Antimicrobial Resistance?

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Quaternary ammonium compounds (QACs) are cationic high-production volume disinfectants and surfactants applied e.g., in hospitals, industry, households and agriculture. As effective broad-spectrum antimicrobials, QAC residues remaining in the environment at sub-inhibitory concentrations could contribute to spreading antimicrobial resistance via co- or cross-resistance mechanisms. This may have been amplified by globally increased disinfectant use during the SARS-CoV-2-pandemic. While wastewater treatment plants (WWTPs) are known emission sources of QACs and hot spots for antimicrobial resistance propagation, comparatively little is known about the use and effects of QACs in agricultural systems. The aims of this study were therefore firstly, to compare the extent of QAC pollution and QAC-tolerance in potentially pathogenic bacteria between livestock farms and human WWTPs and secondly, to investigate how raised disinfectant use during the SARS-CoV-2 pandemic affected QAC concentrations and the abundance of QAC-tolerant bacteria.

Four German livestock farms and three WWTPs were investigated including two farms and one WWTP studied before and after the onset of the pandemic. Manure/slurry and anaerobic digestates of on-farm biogas plants, untreated wastewater, activated sludge, dewatered sludge and treated wastewater (effluent) were studied. Using matrix-specific solid-phase extraction and liquid chromatography-tandem mass spectrometry, 22 different QACs were quantified including alkyltrimethyl, benzylalkyl and dialkyldimethyl ammonium compounds with chain lengths between 8-18 C atoms. QAC-tolerant potentially pathogenic bacteria were cultured on QAC-supplemented agar and their minimal inhibitory concentrations for two QACs and the multidrug resistance (MDR) status were determined.

Our analyses revealed much higher QAC concentrations in WWTPs compared to manure and biogas plant digestates of livestock farms. Although QACs were largely removed from wastewater, they persisted at levels of 2-200 µg g<sup>-1</sup> in the treated sewage sludge and at 0-400 ng g<sup>-1</sup> in biogas plant digestates. The effect of the pandemic was most obvious in dewatered sewage sludge, where concentrations of most QACs increased two- to threefold, but hardly notable in wastewater or farm manure/slurry. The correlation to QAC- tolerance and the MDR status is currently investigated. This study helps to re-assess the ecological and human health risks of QACs in the context of antimicrobial resistance.

#### 4.02.P-Mo312 Effects of the Systemic Fungicide Fluopyram on Aquatic Leaf-Shredders and Microbial Decomposers

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Leaf litter decomposition is an important energy-providing process for heterotrophic stream organisms. Invertebrate shredders mainly drive this process, together with microbial decomposers (i.e., bacteria and fungi). Fungicides might affect decomposition, impairing both shredders and microbial communities. The aim of the present study was to assess how fluopyram affects leaf-associated microbial decomposers and the shredder *Gammarus fossarum* directly by water exposure, indirectly by food exposure, and by the combination of both pathways.

Therefore, *Alnus glutinosa* leaves were conditioned by a field-collected microbial community in the presence and in absence of fluopyram (150 µg/L). We used a 2x2-factorial design, where individuals of *G. fossarum* were exposed to fluopyram directly through the water phase (150 µg/L), indirectly by feeding them with exposed leaves, and in combination of both pathways over 21 days (n=40). Fluopyram effects on microbial decomposers were assessed by quantifying bacterial and fungal operon copies via quantitative real-time PCR. The feeding and excretion rates of *G. fossarum* were assessed every 7 days and growth was assessed at the end of the exposure. Fluopyram concentrations associated with the conditioned leaf material and *G. fossarum* were determined at the end of the conditioning phase and the experiment, respectively.

Results showed that fluopyram non-significantly affected the microbial community by increasing the fungal (~43%) and bacterial (~41%) abundance on leaves, likely accompanied by a shift in the fungal community composition towards more tolerant species that are rejected by shredders, as shown in a previous study. At the same time, fluopyram accumulated in *G. fossarum* through water exposure (5100 µg/kg dw) and the combination of both pathways (5000 µg/kg dw), while showing ~15-fold lower accumulation through feeding exposure only (320 µg/kg dw). Despite these changes at the microbial level and partially high fungicide body burdens, fluopyram did not significantly affect consumption, excretion, assimilation rates, or growth of *G. fossarum* (p>0.05). Although we did not find effects on the feeding behavior or growth of *G. fossarum*, the effects on the microbial community and the high body burden through the water phase and combined exposure point to the potential for long-term effects in freshwater shredder communities that should be studied in further detail.

#### **4.02.P-Mo313 Monitoring of AMR in Environmental Compartments: Considerations from a Regulatory Perspective**

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Following the One Health approach the environment has been recognised as a crucial sector in the development and spread of antimicrobial resistance (AMR). Monitoring or surveillance schemes for AMR and antibiotic use have been developed and implemented most notably in clinical and animal husbandry, but a systematic monitoring for AMR in the environmental compartments e.g., surface and ground water, soil, manure or digestates is still missing. Existing monitoring systems of the health sectors primarily rely on cultivation of pathogens and certain bacterial indicator species of interest. However, these approaches seem to not be directly applicable to the monitoring of antibiotic resistant bacteria or antibiotic resistance genes (ARGs) in the environment.

This is the case, because the majority of environmental bacteria are not cultivable using standard methods. In principle, applying cultivation independent methods like e.g. quantitative PCR (qPCR) allow for the detection and quantification of individual ARGs. Although high-throughput qPCR systems are available which allow measurements of the abundances of a large number of ARGs simultaneously, they still are not yet ready for implementation in routine monitoring, mainly due to high expenditure. To address the regulatory requirement of feasibility, there is a need for prioritisation of ARGs to select a manageable number of highly relevant qPCR targets for standard monitoring of AMR in the environment.

As a first step to develop efficient monitoring strategies in the future, data on present abundances and prevalence of virtually all ARGs occurring in natural environments are gathered to establish background levels of ARGs. In a second step, we present the prerequisites necessary to design efficient monitoring strategies and propose a condensed list of ARGs which are highly relevant for distinct environmental compartments, from a regulatory perspective. The prioritisation criteria comprise e.g., consumption of the antibiotic for humans and animals, selective concentration, and status as critically important antibiotic.

Hereby, we aim at adding regulatory considerations to achieve both, the aggregation of AMR reference data for the majority of environmental compartments as well as a proposed set of ARGs to be used for environmental AMR monitoring.

#### **4.02.P-Mo314 MSC ok? – Experimental vs. Calculated Minimum Selective Concentrations (MSCs) for the Assessment of AMR in the Environment**

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This project aims to close the methodological gap of assessing the risk regarding the spread of antimicrobial resistance (AMR) in the environment within the authorisation of medicinal products. Different approaches are discussed for the computational derivation of so called MSC values (Minimum Selective Concentration) as the basis for their assessment. In these computational approaches, MSC values are calculated from publicly available MIC values (Minimum Inhibitory Concentration). However, so far it has not been experimentally determined which of the approaches is most realistic. Therefore, the aim of this project is to verify the approaches and to subsequently identify the most suitable computational approach for the risk assessment by comparing calculated and experimentally generated MSC values. For this purpose, MSC values will be experimentally determined for an unprecedented number of antibiotics in combination with five environmental bacterial species, respectively. Preliminary data on these comparisons are presented.

Isogenic strains differing exclusively in their fluorescence profile (encoding mScarlet or YFP) were designed from an environmental *Sphingomonas sp.* isolate. The MIC for this strain was first determined for more than twenty antibiotics using a broth-microdilution-assay. Thereafter, the mScarlet encoding strain was allowed to acquire naturally occurring resistance genes for each of the antibiotics through horizontal gene transfer in liquid mating assays with environmental microbial communities originating from soils or wastewater in- and effluents. Then, the resulting resistant strain was grown in competition with the susceptible isogenic strain encoding YFP across a gradient of the corresponding antibiotic. By recording both fluorescence signals

the growth rates of the resistant strain relative to its susceptible counterpart were determined. The MSC corresponds to the tested antibiotic concentration at which the growth rates of the strains are identical.

The experimentally and computationally determined MSC values were then compared for each antibiotic to determine whether existing computational approaches could correctly predict MSC values for environmentally acquired resistances. Finally, the most appropriate computational approach should serve as the basis for developing methodological guidance on how to assess the risk of AMR development and spread in the environment within the authorisation procedure of medicinal products.

#### **4.02.P-Mo315 Detection of Multidrug-Resistant *Staphylococcus* spp. Bound to Plastic Substrates in Surface Water**

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The large and often inappropriate use of conventional drugs in human and veterinary medicine has led to a progressive spread of antibiotic-resistant strains including some important Multiple-drug resistant organisms like MDR *Staphylococcus* spp. In the context of the spread of antibiotic resistance genes (ARGs) and the role of the environmental sector is underestimated in planning prevention action in One health perspective. Furthermore, plastic can become the vehicle for transferring pathogenic microorganisms to other ecosystems, and play a vital role in spreading infections. The current research focuses on implementing the knowledge on the role of aquatic ecosystems in transmitting multi-drug resistant *Staphylococcus* spp and studying its capability of adhering to surfaces submerged in a caldera-type lake of central Italy.

In the late summer of 2021, Artificial Plastic Substrates (APSs) were used to sampling pathogens spread on to aquatic ecosystem. A comparative analysis of the microbial community residing in raw water and colonizing on APS was performed to investigate plastic's potential role in amplifying and spreading pathogenic bacteria, particularly *Staphylococcus* spp. Antibiotic susceptibility was performed using the Kirby-Bauer disk diffusion assay: Tetracycline (TET 30 µg), Gentamycin (GEN 10 µg), Meropenem (MEM 10 µg), Imipenem (IMP 10 µg), Sulfamethoxazole (SXM 25 µg), Ceftazidime (CAZ 30 µg) and Erythromycin (ERY 15 µg). Eight ARGs belonging to three different antibiotic classes; tetracycline (tetA, tetB, tetM, tetW), sulfonamide (sul1, sul2, sul3), β-lactamase (mecA), and two genes for class 1 integrons (intI1 and intI1-V) were investigated by molecular tools. Results. Different types of plastic polymers display different adhesion surface for bacterial colonization; *Staphylococcus* strains were resistant to almost all tested antibiotics. Resistance percentage of isolated strains was as following, TET(80%), MEM(64%), OXA (84%), GEN(88%), CEF (80%), ERY (96%), SMX (92%). Antibiotic resistance genes were found in the isolated strains of *Staphylococcus* with the following percentages: tetA (63%), tetB (86%), tetW (81%), sul1 (31%), sul2 (40%), mecA (100%), intI1 (50%). In conclusion, Considering complex interactions, symbiotic behavior, rising resistance, and infection rates of *Staphylococcus* spp., its colonization on the plastic surface should be strictly monitored to address public health concerns.

#### **4.02.P-Mo316 Mind the Gap: Regulation of Antimicrobial Substances Used in Biocidal Products and Cosmetics**

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Antimicrobial substances are chemicals that can eradicate or slow down the growth of unwanted microorganisms. Upon exposure to antimicrobial substances, the microorganisms are able to rapidly adjust to the new environmental conditions, which may lead to the development of antimicrobial resistance. In EU, different uses of antimicrobial substances may fall under different regulations, each with its own aim, scope and data requirements. Antimicrobial substances used as biocidal active substances in biocidal products fall under the Biocidal Products Regulation (EC) 528/2012. When the same substances are used as preservatives in cosmetic products, Cosmetic Product Regulation (EC) 1223/2009 applies. Both uses involve evaluation and authorization prior entering the EU market, however, different requirements apply. This creates a regulatory inconsistency which may result in unintentional release of hazardous substances into the environment.

We investigated how the regulation of biocidal active substances and cosmetic preservatives focusing on aims, scope, information requirements, and risk assessment procedures. Neither environmental data nor environmental risk assessment were required for approval of cosmetic preservatives. In contrast, environmental data and risk assessment for both the active substance and one representative product would be required for the approval of the same substance if used as a biocidal active substance. The approval of the cosmetic preservatives was not time-limited and once the substance was approved, there was no obligation to update the information on properties, even if such information became available. We also examined the environmental classification of approved cosmetic preservatives under the Classification, Labelling and Packaging Regulation (CLP), and their regulatory status under REACH and the Biocidal Products Regulations. Half of the approved cosmetic preservatives were environmentally hazardous according to classification under the CLP. Some were not approved as biocidal active substances due to the environmental risks and no longer manufactured or imported by the European chemical industry. Despite that, they retained their status as approved cosmetic preservatives. Inconsistencies such as those described above compromise the high level of protection of the environment, and by extension the human health, that EU chemicals legislation aims at.

#### **4.02.P-Mo317 Predicting Selection for Antimicrobial Resistance in UK Wastewater and Aquatic Environments: Ciprofloxacin Poses a Significant Risk**

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The environment acts as a reservoir of antimicrobial resistance (AMR) genes that can be transferred to clinically relevant bacteria. It is increasingly recognised as an important factor in the evolution, dissemination, and transmission of AMR. Antibiotics have been shown to select for AMR at concentrations comparable to those found in the environment. Despite these concerns, they are



released into the environment via wastewater, and this release is not regulated. Further research is required to determine if this release poses a risk that requires mitigation.

To investigate risk of selection for AMR in UK wastewaters, we used measured environmental concentration (MEC) data collected by UK water industry research (UKWIR) in England and Wales 2015-2018. The database contained MECs for four antibiotics: ciprofloxacin, azithromycin, clarithromycin, and erythromycin. We used these data to determine where MECs exceeded minimal selective concentration (MSC) thresholds. There are several methods used to determine MSCs of antibiotics. We used thresholds determined by evolution experiments with a complex sewage community which incorporates community interactions and bacteria likely to be found in wastewater. We also applied a dilution factor of 10 to effluent MECs, to predict concentrations of antibiotics in surface waters.

Results show that selection for AMR by ciprofloxacin is likely to occur routinely in the wastewaters of England and Wales (2015-2018). Wastewater treatment reduced the risk posed by ciprofloxacin significantly, but not completely, with almost 45 % of effluent MECs exceeding a medium selection risk (risk quotient (RQ)  $\geq 1$ ). Additionally, risk was still present in predicted surface waters. We investigated this further to look for seasonal and geographical trends. Although seasonal and geographical differences were observed, instances of high risk (RQ  $\geq 10$ ) in effluent were present at least once across all seasons, and medium risk was present in all geographical locations. Conversely, lower risks were posed by the macrolides (azithromycin, clarithromycin, and erythromycin) and no RQs  $> 1$  were found.

Our data demonstrate risk of selection for AMR is present in UK waste waters and some receiving waters. Further action is needed to prevent selection for AMR, with environmental quality standards for some antibiotics likely required in the future.

#### **4.02.P-Mo318 Antifungal Exposure and Resistance Development: Defining Minimal Selective Antifungal Concentrations and Testing Methodologies**

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Antifungal resistance (AFR) represents a significant, global threat to human health, associated with high mortality rates, absence of effective surveillance systems and with few alternative treatment options available. AFR within the clinic is well documented, though the environment is increasingly recognised to play a role in the evolution and spread of AFR. Diverse fungal communities have been discovered across a wide range of environmental compartments. These communities may be regularly exposed to antifungals from the direct application of effect concentrations of fungicides to agricultural crops and the incomplete removal of pharmaceutical antifungals in wastewater treatment systems, potentially increasing AFR selection risk. At present, environmental risk assessment guidelines do not require assessment of antifungal agents in terms of their ability to drive AFR development, nor are there established experimental tools to determine antifungal selective concentrations. Without data to interpret the selective risk of antifungals, our ability to effectively inform safe environmental thresholds is severely limited. This work aimed to summarise the current understanding of selection for AFR and to compare this with selection for antibacterial resistance. The findings allowed the identification of potential methods to generate antifungal selective concentration data. Data generated by such methods can be considered in the development of regulatory guidelines that aim to reduce selection for AFR in the environment.

#### **4.02.P-Mo319 Microplastics as Vectors of Antimicrobial Resistance in Aquatic Systems**

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Antimicrobial resistance (AMR) is one of the greatest threats to modern society, with the environment recognised to play an important role in the emergence, evolution and spread of AMR. Microplastics are the most prevalent type of plastic pollutant, and frequently co-exist in different environments alongside AMR bacteria, human or animal pathogens, antibiotic resistance genes and antimicrobial residues. Upon entering the environment, microplastics become rapidly colonised by phylogenetically diverse biofilms, also known as 'the Plastisphere'. It is proposed that Plastisphere communities support and enrich distinct, pathogenic and/or AMR communities in comparison to free-living counterparts or communities or those adhered to natural or inert substrates. Due to this, there are concerns that plastic particles may enrich and disseminate AMR pathogens in aquatic systems, potentially increasing human and animal exposure. Using both culture- and molecular-based methods, this research investigated and compared biofilm and free-living bacterial communities following incubation of environmentally aged microplastics and natural or inert substrate controls with a natural sewage community. Selective agar plating and qPCR for 16S rRNA and *intI1* were used to calculate phenotypic resistance prevalence of faecal coliforms and a community wide measure of resistance prevalence, respectively. A pre-described multiplex colony PCR was adopted to identify *Escherichia coli* pathotypes and generate pathotype prevalence within particle and free-living communities. This work identified that surfaces may promote the attachment of AMR bacteria and pathogens, but that the type of surface matters when selecting for these communities. These data will help to develop our understanding of the wider implications of these pollutants on human, animal and ecosystem health.

#### **4.02.P-Mo320 Antimicrobial Residues in Poultry Litter: A Study of its Occurrence in Farms with Different Production Management and Evaluation of a Simple Waste Composting Process**

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In recent years, poultry litter (PL) has gained value under the circular economy perspective due to its advantages as an agricultural fertilizer. However, the use of antimicrobials (AMB) implies the intake of these compounds, which can result in the excretion of up to 90% of the parent compound. The presence of AMB in animal manure poses a risk to both terrestrial and aquatic environments, including the potential spread of microbial resistances among the environmental microbiome. Given the worldwide magnitude of poultry production, and in accordance with the One Health perspective, it is relevant to generate baseline information that contributes to the characterization of the potential risks that this practice may entail to human health. Thus, we analysed the presence of AMB in PL from farms with different management practices. Besides, we performed a typical *in situ* composting process in the studied farms (15 days of stacking with a turning after 7 days). So, we had representative samples to verify the spread of the AMB after PL composting. Considering the complexity of the matrix, different extraction methods were tested, based on: (1) ultrasound and solid-phase extraction or (2) salt-assisted solvent extraction, including 5 different solvents and salts. The optimized method consisted of extraction at pH = 7, using acetonitrile and NaCl. Thereinafter, PL samples from 19 farms were analyzed (n= 3) by UHPLC-QqQ (Waters). Twenty out of 23 studied AMB were detected throughout studied samples at levels ranging from 0.001 mg/kg to 25.3 mg/kg (Sulfamethoxazole). Fluoroquinolones and tylosin were the most frequently AMBs detected, whit ionophores presenting the highest concentrations. Farms that restricted the use of AMB in feed presented less occurrence and load of these compounds in PL. Moreover, we observed that the natural heat, occurring during compost production, lead to a reduction of ca. 50% from the starting concentration in 9 AMB. Our current results highlight the relevance of AMB surveillance in PL residue before deciding its use for composting, manure addition, etc. Finally, reducing the use of AMB into feed and veterinary practices helps with a sustainable use of PL during composting, which may help to reduce the load of these compounds in compost, helping with a sustainable re-use of PL.

#### **4.02.P-Mo321 Removal of Intracellular and Extracellular Antibiotic Resistant Genes (ARGs) by Ultraviolet Light in Different Water Matrices**

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Antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) have been recognized as emerging contaminants of concern. ARGs are known to be persistent and have been detected widely in wastewater effluents worldwide. UV disinfection is gaining implementation at wastewater treatment plants, and could also be used for the removal of ARGs. In this study, different types and forms of ARGs (chromosomal, plasmids; intracellular and extracellular) from different resistant *E. coli*'s were evaluated by UV and UV/H<sub>2</sub>O<sub>2</sub> process as a function of water matrices. The degradation profile of extracellular-ARGs (e-ARGs) showed 1-4 log reductions (90-99.99% removal) during UV treatment. UV-mediated e-ARG degradation strongly depends on the number of active sites and ARG types. The intracellular-ARGs (i-ARGs) showed similar degradation rates as compared to e-ARGs for UV in phosphate buffer saline (PBS). During UV/H<sub>2</sub>O<sub>2</sub> treatment, •OH radicals showed improved degradation for some e-ARGs (1.2-5 logs). However, this advanced oxidation process showed minimal contribution for i-ARG degradation (1-2.4 logs), due to scavenging of •OH radicals by the cellular components in PBS. In contrast to the PBS, the wastewater matrix moderately enhanced degradation, possibly due to the generation of reactive species from effluent organic matter. For the plasmid degradation, conformational differences of the supercoiled structures showed 1.2-2.8 times slower degradation rates than chromosomal ARGs. In addition, the fate of free residual ARGs (*f-ARGs*) and their degradation kinetics were tracked to monitor the correlation between ARB inactivation and subsequent AMR dissemination risk. Overall, these results can be useful for better assessing UV and UV/H<sub>2</sub>O<sub>2</sub> based treatment processes for the effective removal of ARGs from water and wastewater.

#### **4.02.V Antimicrobials in the Environment – A Threat to Environmental and Human Health?**

##### **4.02.V-01 Effects of Erythromycin and Roxithromycin on River Periphyton: Structure, Functions and Metabolic Pathways**

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Macrolide antibiotics have been frequently detected in surface waters worldwide, posing a threat to the aquatic microbes. While several studies have evaluated the ecotoxicological effects of macrolides on single algal and bacterial strains, these results without consideration of the species interaction in the aquatic microbial community cannot be realistically extrapolated to the field. The aim of this study is therefore to evaluate the effects of two macrolides (erythromycin and roxithromycin) on the structure, photosynthetic process, and carbon utilization capacity, as well as the antibiotic metabolic pathways in river periphyton. The colonized periphyton was exposed to the graded concentration (0 µg/L (control), 0.5 µg/L (low), 5 µg/L (medium), 50 µg/L (high)) of ERY and ROX, respectively, for 7 d exposure. Here, ERY and ROX at high level altered the community composition by primarily reducing the relative abundance of Chlorophyta in the eukaryotic community, and the Shannon and Simpson diversity index of prokaryotes were lowered, though similar effects were seldomly detected in the low and medium groups. In contrast with the unchanged carbon utilization capacity, the PS II reaction center involved in the periphytic photosynthesis was significantly inhibited by macrolides exposure. In addition, both antibiotics had been degraded effectively by periphyton, with the removal rate of 51.63% to 66.87% and 41.85% to 48.27% for ERY and ROX, respectively, where side chain and ring cleavage were the main degradation pathways. Overall, this study provides an insight into the structural and functional toxicity and degradation process of macrolides in river periphyton.

##### **4.02.V-02 Metagenomic Analysis Reveals the Spatio-Temporal Shifts of Microbial Community in an Urban River System Contaminated by Macrolide Antibiotics**

**Jiahua Guo**, College of Urban and Environmental Sciences, Northwest University, China

Macrolide antibiotics have been frequently detected in surface waters worldwide, posing a threat to the aquatic microbes. While several studies have evaluated the ecotoxicological effects of macrolides on single bacterial strains, it remains undetermined whether the abundance and biodiversity of bacterial community is negatively linked the occurrence of macrolides in the environment. The aim of this study is therefore to investigate the spatio-temporal distribution of macrolide residues in Zao River (Xi'an city, China), known as an urban river receiving reclaimed water discharged from a Wastewater Treatment Plant, and their potential effects on the aquatic microbial community. Here, the sampling campaign including 20 sites was conducted in dry (Dec, 2021), normal (April, 2022), and wet seasons (Aug, 2022). The levels of five macrolides, including azithromycin (AZI), roxithromycin (ROX), erythromycin (ERY), clarithromycin (CLA), and anhydro erythromycin (ERY-H<sub>2</sub>O) were measured by HPLC-MS/MS coupled with solid-phase extraction. The composition of aquatic microbial community was characterized by metagenomic analysis of the filtered river water. Overall, five macrolides have been detected in Zao river with the concentration of AZI (0 - 1359 ng/L), ERY (0 - 236 ng/L), ROX (0 - 690 ng/L), CLA (0 - 76 ng/L), and ERY-H<sub>2</sub>O (0 to 24 ng/L). Furthermore, the seasonal shifts of macrolide residues are as follows: Dry season > Wet season > Normal season, and the levels were gradually reduced from the outlet to the lower reaches of the river. The redundancy analysis (RDA) between the microbial Operational Taxonomic Unit (OTUs) at species level and macrolide residues indicated that the abundance of bacteria was negatively impacted by the antibiotic residues, including *Opitutae bacterium*, *Verrucomicrobia bacterium*, *Flavobacteriales bacterium*, *Rhodospirillaceae bacterium CCH5-H10*, and *Rhizobiales bacterium KCTC 52945*, in three seasons. In addition, *Chloroflexi bacterium* and *Acidobacteria bacterium* were positively correlated with macrolides in dry and normal seasons, whereas the trend was opposite in wet season. The network analysis is also performed to explore the correlation among antibiotic residues, antibiotic resistance genes, and the structure of microbial community. Overall, this study gains an insight into the distribution characteristics of macrolide antibiotics and their interaction with the aquatic microbial community in an urban river receiving reclaimed water.

#### 4.03.P Biogeochemistry, Ecotoxicology, and Life Cycle of Critical Raw Materials

##### 4.03.P-Tu338 Are Seaweeds Suitable Indicators to Assess Spatiotemporal Patterns in Critical Elements Concentrations? An Insight on the Use of Seaweeds as Biomonitoring in Norwegian coastal Areas

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Rising critical element (including metals and rare earth elements plus yttrium, REY) production and use can also lead to an increasing release into the environment, therefore monitoring the concentrations of these elements in the marine environment is crucial to assess the related environmental risk. Seaweeds are, due to their high abundance, widespread distribution, sessile nature and the capacity to bioconcentrate elements considered as suitable organisms for biomonitoring of element/metal pollution. The aim of the study was to assess the suitability of brown seaweeds as biomonitoring organisms to detect spatiotemporal and source-related contamination patterns of technology critical element concentrations in the Norwegian coastal environment. We also assessed the suitability of two biomonitoring approaches through two case studies. In Case study 1 we applied passive biomonitoring using different species of naturally growing seaweeds specimens and in Case study 2 we used active biomonitoring, using cultivated specimens of a single seaweed species (*Saccharina latissima*) deployed at specific monitoring sites. Element concentrations were determined using ICP-MS on freeze dried seaweeds tissue samples. Relationships between element accumulation, and presence of local point-sources, environmental and biological parameters were evaluated. Results from Case study 1 indicated spatial and species-specific differences in REY concentration according to the sampling site, with seaweeds (specially *Fucus* spp. and *Saccharina latissima*) sampled in proximity to potential anthropogenic point-sources having the highest REY concentrations. Findings from Case study 2 showed a high spatiotemporal variability in element concentrations, with different elements (or element groups) exhibiting specific trends. REY behaved as a coherent group, showing similar spatiotemporal trends with accumulation being most pronounced in a freshwater impacted site. Other investigated elements exhibited various spatiotemporal trends according to different biotic and abiotic drivers. Our findings indicate that (either natural or cultivated) seaweeds can act as useful biomonitoring organisms for assessing spatiotemporal variation of the bioavailable dissolved element fraction as well as to assess the presence of local anthropogenic element sources. Furthermore, species-specific difference in element uptake and accumulation patterns exists and need to be considered for future use of biomonitoring.

##### 4.03.P-Tu339 Fate and Biological Effects of Rare Earth Element mixtures in a Model Freshwater Food Web

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The growing uses of rare earth elements (REE) led to increasing releases of anthropogenic REE in freshwater systems from effluents of mining, industrial, medical, wastewater treatment plant (WWTP) activities, which raise concern about their environmental risk. The lack of environmental realism in REE ecotoxicological studies implies a limited extrapolation of the results to natural ecosystems. The objectives of this study was to i) assess REE mixture (Nd+Gd+Yb) fate and biological effects in more realistic conditions reproducing environmental scenarios of REE contamination: WWTP effluents and industrial/mining

activities, set at 15 and 1500  $\mu\text{g L}^{-1}$  total REE concentration, respectively, ii) compare the fate, bioaccumulation and toxicity of Gd in its chloride form (GdCl) and MRI contrast-agent (GdCA), iii) assess the potential REE trophic transfer.

We reproduced a freshwater food web in indoor mesocosms using the primary producers *Myriophyllum spicatum*, *Raphidocelis subcapitata* and *Chlorella vulgaris*, the primary consumers *Corbicula fluminea* and *Daphnia magna* and the secondary consumer *Danio rerio*. After 28 days of exposure, we measured a battery of biomarkers including growth, behaviour, oxidative stress, cellular damages, elemental homeostasis, photosynthetic pigments on *C. fluminea*, *D. rerio* and *M. spicatum*. In addition, REE were quantified in the medium and in the organisms.

The results showed that species from all trophic levels accumulated significant concentrations of Nd, Gd and Yb. REE bioaccumulation was not influenced by food contamination and decreased with increasing trophic level demonstrating the limited trophic transfer of REE. Exposed bivalves and fish showed increased activities of GST, LDH and CSP-3 activities suggesting a stress and potential tissue damages. Osmoregulation was disturbed in exposed bivalves with an inhibition of  $\text{Na}^+\text{K}^+\text{-ATPase}$  activity in *C. fluminea*. *M. spicatum* growth was inhibited and photosynthetic pigment contents decreased at the highest tested concentration. GdCA had an opposite fate compared to GdCl with a lower bioaccumulation and a different internal distribution in *C. fluminea* and *D. rerio*.

Overall, this study showed that current effluents from mining, industrial and WWTP activities are of concern because they contain high concentrations of REE that can be accumulated and induce toxic effects on species belonging to different trophic levels, especially primary producers.

#### 4.03.P-Tu340 Bioavailability and Effects of Different Forms of Gadolinium in Three Fish Species

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Gadolinium is one of the rare earth elements (REY), and is, amongst others used as contrast agent for magnetic resonance imaging (MRI) diagnostics. Due to toxic properties of  $\text{Gd}^{3+}$  Gd-based contrast agents (GBCAs) are administered as Gd-chelates. GBCAs are not retained in wastewater treatment plants and are thus released into the aquatic environment where Gd can be detected as micropollutant in rivers, groundwater, and coastal waters. Gd emissions from MRI imaging are substantial and are estimated to several tons annually. However, knowledge on the effects of Gd on aquatic organisms is still scarce. In the Elementary project we studied effects of inorganic Gd ( $\text{GdCl}_3$ ) and GBCAs on three fish species: embryos and larvae of zebrafish (*Danio rerio*), embryos and larvae of Atlantic cod (*Gadus morhua*) and juvenile lumpfish (*Cyclopterus lumpus*).

Our results show that, while Gd is bioavailable in both forms,  $\text{GdCl}_3$  is taken up to a larger extent in early life stages of fish.  $\text{GdCl}_3$  exposure did have negative effects on both zebrafish and cod larvae. However, effects were more pronounced in cod, where we found early hatching, an increase in mortality and deformation rates as well as lacking pigmentation following exposure to (nominally) 300 and 3000  $\mu\text{g/L}$  Gd equalling approximately 130  $\mu\text{g/L}$  of dissolved Gd as analysed by ICP-MS. In contrast, these concentrations did not affect survival or development in zebrafish, showing a lower sensitivity in comparison to cod. We did, however, find effects on neural activity in form of a decrease of pERK and tERK in the brain of zebrafish exposed to  $\text{GdCl}_3$  at high concentrations. In contrast, GBCA caused a slight increase in neural activity.

In lumpfish, exposure to low concentrations of  $\text{GdCl}_3$  and GBCA did not cause mortality or obvious behavioural changes. Similarly, no significant effects on body mass and the determined blood parameters. Uptake, organ distribution and effects are analysed.

Results of our study show that free Gd can affect early life stages fish, and show a greater sensitivity of cod compared to zebrafish. Our findings highlight that effects of chemicals can be overlooked in standard toxicity tests, and that a more in-depth investigation, also including potentially exposed non-standard species can be advisable. While we did not observe effects of the tested GBCA, data on GBCAs stability in the marine environment are scarce and await further studies to determine environmental fate and potential effects.

#### 4.03.P-Tu341 Deep-Sea Mining as an Emerging Stressor in Deep-Sea Environments: Case Studies Using Hyperbaric Chambers for Hazard and Risk Assessment

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The deep-sea, the largest biome on Earth, despite its remoteness is subjected to anthropogenic stressors. These stressors include, among others, deep-sea mining, an emerging threat to deep-sea environments. Deep-sea mining is being looked at with renewed interest due to the increased need for minerals and rare earth elements that are depleting land-based deposits because of technological advances, mainly in green energy. Deep-sea mining will release sediment plumes, clouds of resuspended particles, considered one of the most extensive and immediate effects of mining as they can travel hundreds of kilometers across the water column.

To study the potential effects of suspended sediments, we used two model species, the *Mytilus galloprovincialis* mussel and the *Spisula solida* clam, exposed for 96h in a hyperbaric chamber to different size classes of sediments (63-125;125-250;250-500 $\mu\text{m}$ ) in a mixture with different concentrations (1,2 and 4g/L) at increasing pressures (up to 50Bar). We analyzed the filtration rate

(FR) and oxidative stress biomarkers in juvenile mussels and adult clams. The FR decreased in all conditions and significant changes were observed in all tested biomarkers, which were concentration and pressure dependent. We also studied the effects of the different size classes of sediment separately by exposing juvenile mussels to the different sizes of sediments at 1g/L and 4Bar. The FR, molecular and biochemical endpoints were analyzed. The FR decreased significantly for all sizes with a more pronounced effect in the smaller particles. Significant changes were found in all tested biomarkers and gene expression was altered in some genes related to oxidative stress (catalase and actin).

Sediments affected the organisms at all levels, with a more pronounced effect for the smallest particles. As these particles are known to have high dispersion capacities, particular attention should be given to their release as a by-product of mining. These findings will help to fill knowledge gaps in the effects of suspended particles in deep-sea environments, contribute to hazard and risk assessment of deep-sea mining and to the establishment of frameworks to mitigate the negative effects of this stressor in sensitive habitats.

#### 4.03.P-Tu342 Potential Future Ecotoxicological Problems in Soil Irrigation by Gadolinium Pollution

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Rare earth elements use has increased in the last decades in multiple anthropogenic activities from green energy and medical technologies to telecommunications. However, due to until the end of last century they had never been characterized as essential or toxic for living organisms, the current understanding on the concentration, behaviour and possible ecological effects of almost all REEs is limited, and no regulatory information has been settled. One of the most frequently detected anthropogenic REE is gadolinium (Gd), the occurrence of Gd anomalies is highly correlated with the use of this metal in medical applications, especially in densely populated areas. For example, it was reported that the release of Gd in Germany, related to biomedical purpose, was ca. 12t Gd per year. Since Gd cannot be removed by common sewage treatment technologies, we expect that it will be easily increase their accumulation in irrigated soils. Therefore, the aims of the present study are to (1) perform a preliminary ecotoxicological risk assessment for Gd in soils; and (2) assess the potential trophic movement of Gd in a crop irrigated with water enriched in this element. For this study soils with contrasting properties, will be spiked with different Gd concentrations (from 0 to 4000 mg kg<sup>-1</sup>) and short-term ecotoxicological assays with lettuce, due to is a well-known laboratory model and an irrigated crop, will be performed. According to the EC values obtained in the ecotoxicological test, and the concentrations of Gd in water rivers of the city of Granada, potential Gd content in irrigated waters will be selected. Different soils will be irrigated and incubated for at least 4 weeks. After that, a lettuce plantation will be culture (28 d) and irrigated weekly with Gd enriched water, to check the toxic effect and potential Gd accumulation and translocation in plants. Our results will shed light on the effect of use waters with anthropogenic Gd in irrigating crops and if the continuous discharge of this element in aquatic systems could lead to plant detriment and, finally, pass Gd through the trophic chain.

#### 4.03.P-Tu343 Evaluation of the Effects of a Mixture of REE on Gene Expression of *Daphnia magna*

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Rare earth elements (REE) are considered as technology critical elements. Compared to trace elements with well-documented environmental impacts, REE remain less studied, and their toxicity mechanisms are not fully understood. A transcriptomic approach through a targeted gene expression method was used to investigate REE effects on *Daphnia magna* at sub-lethal concentrations. A set of stress-related genes was selected to assess the effects of a mixture of REE on *D. magna* juveniles. In this study, *D. magna* were exposed for 21 days to up to 600 µg.L<sup>-1</sup> to a mixture of Gadolinium (Gd), Neodymium (Nd) and Ytterbium (Yb). At the end of the exposure period, juveniles from the fifth brood were kept and exposed for 48h to up to 600 µg.L<sup>-1</sup> to REE mixture (Gd + Nd + Yb). Effects of REE on the expression of a set of stress-related genes have been evaluated using RT-qPCR. Genes involved in apoptosis, osmoregulation, energy metabolism, antioxidant defense, metallic stress, digestion... etc. have been assessed

A concentration-dependent effect was observed on *D. magna* gene expression. The higher concentrations of the REE mixture led to an up-regulation of genes involved in general stress responses (e.g. HSP70, Glutathione-S-Transferase), apoptosis (P53, Caspase 8), energy metabolism (Fructose-bisphosphate aldolase, phosphofructokinase, and GAPDH), and osmoregulation (Na<sup>+</sup>K<sup>+</sup>-ATPase). Our results on the gene expression of *D. magna* suggest that REE do not impair a specific pathway but seems to have generalized impacts. The large number of candidates genes and proteins identified by the omic approaches applied for this study provides a solid starting point for further findings to identify REE mechanism of action towards aquatic organisms.

#### 4.03.P-Tu344 Protective Role of Colloids against Cr and Ni Ecotoxicity in a Small Ultramafic Catchment

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In ultramafic environments, nickel (Ni) and chromium (Cr) often exceed the corresponding environmental quality standards (4 µg/L as bioavailable Ni) or guideline values (e.g., 10 µg/L for Cr(III) and 4 µg/L Cr(VI) according to WHO). Water samples were collected in a small ultramafic creek (Pluhuv Bor, Czech Republic) located far from anthropogenic sources of trace elements other than atmospheric deposition. Sample collection was carried out in December 2021 during the rising limb of a small flood with water discharge increasing from about 0.2 L/s (baseflow) to 2.6 L/s. Filtered waters (0.22 µm) were analyzed for Ni and Cr content and tested for ecotoxicity with the freshwater alga *Raphidocelis subcapitata*. Filtered waters had concentrations of Ni (80-

190 µg/L) and Cr (10-23 µg/L) above the corresponding ecotoxicological threshold values, but did not adversely affect the growth of *R. subcapitata*. Analysis of ultrafiltered water aliquots (3 kDa) showed that 60 to 95% of the filterable Ni and Cr were associated with the operationally defined colloidal fraction (3 kDa–0.22 µm), suggesting a possible protective effect of colloids against Ni and Cr ecotoxicity. Both total filterable concentrations and the colloidal Ni and Cr pools increased with flow, while concentrations < 3 kDa remained constant at around 25-30 µg/L for Ni and 1.3-1.6 µg/L for Cr. In situ measurements of Ni and Cr levels by DGTs (Diffusive Gradients in Thin Films) were in excellent agreement with ultrafilterable concentrations. In the case of Cr, DGT-labile concentrations mainly occurred as Cr(VI), while ionic Cr(III) levels were close to analytical detection limits. Measurements by ion-chromatography high-resolution ICP-MS gave ionic Cr(VI) and Cr(III) concentrations equivalent to those determined by DGTs, but also highlighted the presence of an unknown Cr species. Ecotoxicity tests with addition of Cr(III) and Cr(VI) to filtered waters showed no toxicity to *R. subcapitata* for concentrations up to 0.9 mg/L of Cr(III), while the EC<sub>50</sub> of Cr(VI) increased 4-fold compared with results obtained in standard ISO medium for freshwater algae. Overall, the presence of colloidal carrier phases (likely Fe oxides and natural organic matter) seems to play an important role in limiting Ni and Cr bioavailability and ecotoxicity to algae in freshwater ultramafic environments. The possible contribution of colloidal Ni and Cr to elemental bioavailability for e.g., filter feeders deserves further investigation.

#### 4.03.P-Tu345 Microcosms as a Tool to Study REE Toxicity in a Simulated Aquatic Food Web

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Rare Earth Elements (REE) show high adsorption affinity for sediment particles and fine organic matter, making it likely that benthic organisms are the species most exposed to REE in the environment. Resuspension of sediment or exchange of porewater may mobilize REE and re-introduce it to the water phase. This benthic-pelagic coupling might also lead to effects on pelagic as well as benthic species. Indoor microcosms are artificial and simplified ecosystems that under controlled conditions aid in the research of the effects of contaminants and stressors in the natural systems. This makes microcosms highly suitable for our objective to investigate the impact of sediment resuspension on the transfer of lanthanum (La) and gadolinium (Gd) from benthic layer to the pelagic layer at the first consumer level. The objective for this study is to combine the information gathered from previous studies and add a deeper insight in the role of sediment re-suspension on the aquatic food web. The 2 L salinized glass microcosms used in this study will contain five aquatic grown laboratory species; daphnia (*Daphnia magna*), microalgae (*Raphidocelis subcapitata*), nematodes (*Caenorhabditis elegans*), bacteria (*Escherichia coli*), and ostracods (*Heterocypris incongruens*). The microcosm will be set-up in four gradual steps, in order for the ecosystem to stabilize with each addition: 1) bacteria and algae are added to a layer of uncontaminated sediment and media, 2) nematodes and daphnia are added to the established bacteria and microalgae populations, 3) introduction of the ostracod population, and 4) resuspension of sediment. We will look at parameters such as death rate (EC<sub>50</sub>), reproduction rate (RC<sub>50</sub>), population count, according to the guidelines of the bioassays related to the species. The microcosms will be spiked with (nominal) environmentally relevant (1 and 10 µg L<sup>-1</sup>) and anthropogenic concentrations (100 and 1000 µg L<sup>-1</sup>) of La and Gd. Our hypothesis is that benthic organisms higher will be more impacted by a REE-supply to the “environment” than pelagic species or primary consumers. From previous studies it is also most likely that Gd is more toxic than La. We will further elaborate on this study and present preliminary results in the poster.

#### 4.03.P-Tu346 Leaching of Elements from Smartphones and their Toxicity to Marine Organisms

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Rapid development, increasing demands and short lifespans of electronic equipment causes high generation of electronic waste (e-waste). In 2019 alone, approximately 1.5 billion smartphones were sold world-wide, with around 1.7 million smartphones sold in Norway. Mobile phones, especially smartphones are composed of different materials, including toxic metals such as nickel, copper, zinc, silver, cadmium and lead, several of the rare earth elements (praseodymium, gadolinium, terbium, dysprosium) and organic chemicals, e.g., flame retardants and perfluorinated chemicals. E-waste can thus pose an environmental threat, when not properly handled. In this study we conducted a first investigation on the leaching of elements, including heavy metals and rare earth elements from smartphones into seawater. We further studied effects of leachates on marine species from three trophic levels. Four mobile phones of the same brand and similar type were shattered and incubated in natural, seawater under gentle agitation (20 °C, darkness). Water samples are taken after 1 week, 1 month and 2 month and were analysed for elemental concentrations with ICP-MS. Incubation in seawater resulted in a rapid release/formation of large amounts of fine particles. Results show that after two month, the mobile phones leached significant amounts of the major elements like Na, Mg, Si, K, and Ca, but also significant amounts of Al, Fe, Ni, Zn, Ge, Sr, Mo, Sn. Further, we detected La, Nd and Pr from the group of REYs. Results further showed that both the dissolved (particles removed with centrifugation) and total (particulate and dissolved) fraction of the 1-week leachate inhibited the growth of the marine algae *Skeletonema pseudocostatum*, with the total fraction causing more pronounced effects. Further, the total fraction increased mortality in the marine copepod *Acartia tonsa*. While we did not see effects of most leachates, the leachate of one of the mobile phones caused a 70 % mortality in exposed cod embryos. Further studies are needed to identify which elements or potentially other contaminants are responsible for the observed toxic effects.

#### 4.03.P-Tu347 Rare Earth Element Pollution Recording in the Saltwater Mussel *Mytilus* Spp. in Norwegian Coastal Waters

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Growing extraction and usage of rare earth elements and yttrium (REY) in different industrial sectors has culminated in their increased discharge into the marine environment. In particular, globally observed anthropogenic enrichment of gadolinium (Gd) in aquatic systems near populated areas is due to its use as a magnetic resonance imaging (MRI) contrast agent. This study aimed to analyze the accumulation, spatial distribution, and shale-normalized patterns of REYs in *Mytilus spp.* soft tissue at a potential release hot-spot in southern Norway. Mussel specimens (n=49) were collected at three sites around a spillwater pipe of an industry producing Gd-based MRI contrast agents (GBCAs) and compared to a reference site. Results show that mussels at an enclosed bay in the vicinity of discharge point accumulated significant, over fourfold higher total mean REY (4.88 µg g<sup>-1</sup> d.w.) in its soft tissue in comparison to other locations and the reference site (0.95 – 1.08 µg g<sup>-1</sup> d.w.). Normalization with European shale highlighted positive Gd anomalies in all sites, including the reference. However, a notable fourfold Gd anomaly (Gd/Gd\* = 4.4) was observed in the mussels downstream of the GBCA industry outfall, indicating a clear excess uptake of anthropogenic Gd by mussels in this area. The mussels at the enclosed bay, despite carrying the highest total REY concentrations, retained a similar Gd anomaly with the reference site. Moreover, our results show that mussel soft tissue in southern Norway is generally characterized by an enrichment of LREYs over HREYs, with LREYs contributing to 82 ± 4% of total REY burden. The three metals representing the majority of REY concentration were Ce (33 ± 3%), La (26 ± 4%), and Nd (16 ± 2%). Among the heavy REYs, Y contributed the most (10 ± 1%) to the total REY burden in mussels. This study constitutes the first record of Gd excess and REY accumulation in marine bivalves in Norway, and will be useful for future biomonitoring studies on REY contamination.

#### 4.03.P-Tu348 Tissue-Specific Bioaccumulation of Rare Earth Elements in Brown Crab (*Cancer Pagurus*) at an Industrially-Affected Fjord in Porsgrunn, Norway

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The critical use of rare earth elements and yttrium (REY) in a wide range of emerging applications has led to their release in aquatic systems through urban and industrial wastewater runoff and rivers. Benthic organisms near industrial zones can be increasingly exposed via contaminated substrate or diet, yet information is still limited regarding REY bioaccumulation and organotropism in marine biota. In this study, we investigated the tissue-specific accumulation and profile of REYs in a commercially important marine species, the edible brown crab *Cancer pagurus*. Mature female crabs (n=18) were taken from three sites around Frier fjord in Grenland district, a heavily industrialized region in southern Norway. From each crab, the hepatopancreas (brown meat), claw muscle (white meat), and gills were dissected, and REY concentrations were determined using ICP-MS. The results show that *C. pagurus* accumulated majority of REYs burden in gills (17 - 69 µg total REY g<sup>-1</sup> d.w.), and much less in hepatopancreas (0.69 - 1.2 µg g<sup>-1</sup> d.w) and muscle (0.23 - 0.58 µg g<sup>-1</sup> d.w). Using the results from the gills, we assessed the spatial distribution of REY concentrations in Frier fjord. Crabs in the sites closer to the Knardalstrand wastewater treatment plant discharge point and the Skien river outlet had three to four times higher total REY concentrations than those in the reference site 8 km away. European-shale normalized REY patterns showed that all tissues exhibited an enrichment of light REYs over heavy REYs, and small positive Gd anomalies (Gd/Gd\* = 1.07 - 1.26). La, Ce, Nd, and Y were the most abundant REYs across all three tissues. The percentage of La in claw muscle was significantly higher than in the gills and hepatopancreas, while for Ce the hepatopancreas had significantly lower percentage than the in gills and claw muscle. This study provides the first report of REY accumulation and organotropism in brown crabs, and demonstrates the use of crab gills as a sensitive organ for future biomonitoring of REY contamination.

#### 4.03.P-Tu349 Are Technologically Critical Metals (Rare Earth Elements, REE) Accumulating in Harbour Porpoise (*Phocoena phocoena*) from Norwegian Coastal Waters?

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Harbour porpoises (*Phocoena phocoena*) are the most abundant cetacean in Norwegian waters. Rare earth elements (REE) are essential components in a wide range of products and applications, including innovative and green technologies. This causes a rapidly rising production and use of REEs, leading to their release into the environment - with so far unknown consequences. Being a part of the Fennoscandian shield, Norway has high potential for REE exploration and production. The potential toxicity of REEs and their accumulation in marine mammals is not well documented. The aim of this study was to determine REEs concentrations in liver and muscles of harbour porpoise and examine their spatial distribution in relation to potential sources. In addition, the influence of age and sex on the accumulation of REE in porpoises was examined. In 2016 and 2017, 134 specimens were collected along Norwegian coast. REEs (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Pm, Sm, Tb, Tm, Yb), Sc, Y, U and Th were quantified in the muscles and livers by HR-ICP-MS. Animals were divided into different locations, corresponding to fishing areas. Highest hepatic concentrations for Ce, La, Nd, Pr and Y were found in individuals from Grøtsundet and Varangerfjorden, northern Norway. This may be attributed either to increased mining of minerals that contain various complexes of REEs or to natural geological differences in bedrock composition. Hepatic Ce, La, Nd, Pr and Sm concentrations were positively correlated with body size, indicating accumulation of these metals with age. No sex differences in accumulation of REE were found, suggesting lack of reproduction elimination mechanism for these metals in harbour porpoise. This study provides important background data for REE in top predators in marine environment for future studies on anthropogenic pollution which is expected due to high demand for REEs in modern technological applications.

#### **4.03.P-Tu350 Rare Earths and Other Elements in Transplanted Blue Mussels (*Mytilus edulis*) and Seaweeds (*Saccharina latissima*) Near Salmon Farms: First Results from a Site in Trøndelag, Norway**

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Intensive sea-based Atlantic salmon (*Salmo salar*) farming is a source for multiple stressors to the marine ecosystem, such as parasites and diseases, contaminants including (heavy) metals, microplastics, pesticides and other organic pollutants, and excess nutrients. Many of these pollutants are directly associated with salmon feed, which is released and spread via the open net-pens either as uneaten pellets or faeces. Recent analyses have revealed that the technology-critical rare earth elements and yttrium (REY), which were lately classified as contaminants of emerging concern in aquatic systems, can be found in salmon feeds at levels exceeding background seawater concentrations by about 4 orders of magnitude. REY tend to bioaccumulate especially in lower trophic level organisms such as invertebrates and algae, with toxic effects at surplus levels. The magnitude of potential REY emissions from salmon farms and their relevance as contaminants in adjacent ecosystems have not been considered before, yet, this knowledge is crucial for identifying the risks of REY to marine wildlife.

Here, we will present the first findings from a four-month screening of REY at a salmon farm in Trøndelag, Norway. We transplanted both blue mussels (*Mytilus edulis*) and sugar kelp (*Saccharina latissima*) seeded twines from February to June 2022 near the salmon cages (S1: at the cages, S2: around 50 m downstream in the main current direction) using three replicates per station. As controls we used mussels sampled at time zero and seaweeds that were farmed since January in the same area, but > 1 km away from all fish farming activity. After retrieval, biometric measures were taken and the mussel soft tissues as well as total sugar kelp fronds were deep-frozen, freeze-dried, homogenised, digested and are currently analysed for REY and other relevant elements using ICP-MS. The results will provide novel insights into the potential spread of REY with salmon aquaculture wastes to the marine ecosystem and species-specific bioaccumulation patterns of REY and other elements.

#### **4.03.P-Tu351 Toxicity of Three Rare Earth Elements on the Deep-sea Scavenging Amphipod *Tmetonyx cicada* (Lysianassidae): Linking Molecular and Behavioural Responses**

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In response to a sharp rise in demand for the technology-critical rare earth elements (lanthanides) and yttrium (together: REY), Norway has recently announced plans for deep-sea mining of these metals. In other parts of the world, REY mining has led to emissions of environmental concern. Recent research points out their persistence and enrichment in aquatic sediments, potential for bioaccumulation and toxicity to wildlife. Existing studies indicate coherent toxicity and modes of action across the group of REY, likewise, REY toxicity has been shown to vary across study organisms and with dependence on atomic weight. By interference with key physiological pathways such as cellular Ca<sup>2+</sup> signalling, REY have the potential to cause a broad range of toxicological effects, from neurotoxicity, over developmental and cardiovascular effects, geno- and proteotoxicity to lethality at high levels. Yet, the underlying effect mechanisms are insufficiently understood. Moreover, most existing studies were performed on model species, many of which showed low sensitivity to REY. Environmental relevant ecotoxicological data, especially for the marine ecosystem, are scarce but urgently needed to assess the risks of REY.

In this work we studied the effects of three REY on the health of the lysianassid amphipod *Tmetonyx cicada* (Fabricius, 1780), a locally abundant scavenger that inhabits the deep seafloor from the North Atlantic up to the Arctic. *T. cicada* were collected in spring 2022 from Saltfjorden (Bodø, Norway) at 180 m depth using herring-baited traps. Not fully matured specimens of 1-2 mm size were exposed for 10 d to a light, medium and heavy REY (Nd (144.24 g/mol), Gd (157.25 g/mol) and Yb (173.04 g/mol)), at concentrations of 0, 3, 30, 300 and 3000 µg L<sup>-1</sup> in a dark climate cabinet at 8 °C. We observed mortality for all three REY at 3000 µg L<sup>-1</sup> and increased swimming speed under a white-light stimulus at sublethal concentrations. Transcriptomic analyses will further provide insight to underlying molecular modes of action. Our results so far indicate that REY mining in Norway may adversely affect the marine ecosystem, as aquatic REY loads downstream of existing mining sites have been shown to reach several mg/L up to g/L. This emphasises the need for a risk assessment of REY and establishment of a regulatory framework to prevent toxic REY releases from existing sources and future mining sites.

#### **4.03.P-Tu352 Effects of Cu Exposure on Atlantic Cod (*Gadus Morhua*) Embryos and Larvae**

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While copper (Cu) is a natural constituent of the environment it is also a toxicant for aquatic species. Due to increased demand for Cu (up to 50% over the next 20 years), its levels in areas with high anthropogenic activities are of concern, especially in mining and deposition areas. Deposition of tailings on the seafloor, as practiced in several sites along the Norwegian coast, releases particles to the marine environment that may contain both process chemicals and heavy metals.

Atlantic cod is a commercially important fish species that spawns in coastal areas. The pelagic larvae can be retained in fjord systems for long periods of time, making them a potential target for marine tailing exposure.

In this study we exposed Atlantic cod embryos (6 replicates) from 3 days post fertilization (dpf) to 4 days post hatch (dph) to three different Cu concentrations: 5, 15 and 30 µg/L Cu, and a control. Biometric images were taken, and respiration was measured daily. Samples were also collected for differential gene expression and metabolomic analysis at two time points, one for embryos (96 h) and one for larvae (2 dph).



Embryos in the high treatment group hatched earlier and total mortality was significantly higher compared to all other groups. While no significant difference was found in respiration, larvae in the high treatment group were smaller and had higher deformation rates. Transcriptomic profiling and metabolomics analysis revealed a higher expression of stress related genes and a significant increase in oxidized glutathione (GSSG) in embryos and larvae from the high treatment group which is indicative of oxidative stress. Also, changes in metabolites related to energy metabolism and upregulation of genes related to haemoglobin transport and metabolic rate suggests an active detoxification process in larvae from the high treatment group which could help explain the slower growth of larvae.

Our result show that early life stages of Atlantic cod is sensitive to environmentally relevant levels Cu around marine tailing deposition sites.

#### **4.03.P-Tu353 Effects of Short-Term Mine Tailing Exposure on Stage CV *Calanus Finmarchicus***

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Mineral demand for new technologies and industries drives an increase in mining activities worldwide. As tailing deposition on land takes up large areas and give rise to numerous environmental challenges, marine tailing disposal has been proposed as an alternative in some areas.

The marine filter feeding copepod *Calanus finmarchicus* is an important food source for commercially important fish species and other marine animals due to its high lipid content. The release of particles and chemicals associated with marine tailing deposition may pose a potential threat to fat-rich zooplankton, as previous study revealed a delay in development and lower lipid accumulation in developing *C. finmarchicus* exposed to mine tailings.

To investigate the potential short-term effect of mine tailing exposure on later life stages, *C. finmarchicus* stage CV was exposed to pure calcium carbonate particles and Cu in high and low concentrations, as well as calcium carbonate and copper tailings in low, medium, and high concentrations, with five replicates per group, for 96 hours (10 °C). In addition, five replicates of control (clean sea water with food) and starved control (clean sea water with no food added) were included. At termination, respiration measurements and biometry images were taken, and samples for metabolomic analysis were collected.

The results showed that the lipid reserve was lower in all treatment groups compared to the control, and significantly lower for the high calcium carbonate particles and tailing treatments and the starved control. Respiration rates in animals from low tailing and high and low Cu exposures were higher than respiration rates in the control group, while medium and high tailing exposures generally caused lower respiration rates. The lowest respiration rates were observed in animals from the starved control group.

The global metabolic profiling results revealed changes across a broad range of pathways, including mechanisms linked to energy metabolism and growth. One of the most striking changes occurred in the glutathione pathway, which is highly consistent with previous work demonstrating the role of reactive oxygen species (ROS) and redox imbalance in starved animals.

Our result suggests that ingestion of tailing particles most likely impair metabolism and induce a response resembling starvation in copepods exposed to mine tailings.

#### **4.04 Bioremediation and Phytoremediation of Aquatic and Terrestrial Contaminated Ecosystems**

##### **4.04.T-01 Increasing the Removal of Micropollutants in Municipal Wastewater by Natural Adsorbents Addition in Constructed Wetlands: Road to Circular Economy**

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The occurrence of micropollutants (MPs) in aquatic ecosystems are mainly attributed to the discharge of wastewater from conventional wastewater treatment plants (WWTPs), which are not specifically designed to eliminate them. Even at low concentrations, many of these compounds can cause hazardous effects on human health and ecosystems. Thus, additional treatments are required at WWTPs to eliminate these MPs. Although some processes have been tested, research on Nature-based Solutions (NbS) is limited investigated.

Among NbS, constructed wetland (CW) is one of the technology recognized for their ecofriendliness, low costs of operation and maintenance and simplicity. However, the requirement of large time of operation, since are natural treatments, could limit the implementation of CW on a full scale. In this sense, this work aimed to investigate the performance of addition of various natural support matrices (two of them wastes of food industry) since they present high adsorption properties, in CW for their capacity to remove 27 selected MPs at 1 µg/L in secondary wastewater effluent. With this purpose, it was intended to reduce the time of operation in CW by the addition of potential adsorbents and framing the project in circular economy.

In this study, four vertical flow CW (VFCW) planted with *Sparganium erectum* were assembled at semi-pilot scale (15 L water capacity) with recirculation. Each one was filled with a layer of cobbles, volcanic rocks, fine gravel and sand. In three of them, an additional material mixed with sand was added (burnt cork, almond shell and chestnut shell). The last one, without any additional material was used as control.

The results at 6 days of retention time revealed good performances of VFCW filled with almond and chestnut shell reporting a MP elimination in water higher than 90% for overall compounds (25 of 27). VFCW containing burnt cork and control showed the lowest removals (only 15 and 13 of 27 MPs, respectively, achieved eliminations higher than 90%). The total average abatements were: 88, 87, 77 and 68% for chestnut, almond, burnt cork and control, respectively. Comparing 3 and 6 days of RT the results were very close in systems containing chestnut and almond shell. For instance, in VFCW with chestnut shell the average removal

was only increased 7% from 1 to 6 days. However, in control CW the differences increased by 18%. This data revealed the possible reduction in operation time when additional material was added to CW.

#### **4.04.T-02 Towards the Transformation of a Contaminated Industrial Wasteland into a Living-lab Based on a Phytomanagement Approach**

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The main goal of the ECOPOLIS project is the restauration by phytomanagement of the industrial wasteland of Vieux-Charmont (Doubs, France), located in an urban area and contaminated by metallic trace elements (TE) and hydrocarbons. With the collaboration of different public and private partners, a living-lab will be implemented on the area for research activities and communication with the public. This implementation is based on three specific objectives, (1) to characterized spatially and quantitatively the contamination, mobility, health and environmental impacts due to contaminants on the site. This will be done by monitoring biodiversity on the site and measuring contaminants transfers into ground and surface water, vegetation and animals, (2) To test different management scenarios for the contaminants based on phytoremediation methods in accordance with the principles of the National Methodology for the Management of Polluted Sites and Soils and its associated standard (NF X31-620) and (3) To assess the potential reproducibility of the methods on similar industrial wasteland though the territory. In this presentation, we will address objective 1 mostly. The presentation will focus of the data sets obtained within the period 2020-2022. The first set of data showed that 3 TE are dominant on the site, Zn, Pb and Cd, that have been shown to have potential toxic effect on human health, based on the exposure scenarios and bioaccessibility tests. A botanical survey led to the identification of some native plants that can accumulate Zn (> 2000 mg/kg DW) and Cd (> 15 mg/kg DW) in aerial parts. Some of these plants are further being studied for their phytoextraction potential, with the final aim to develop tools to reduce the available contents of these elements in the soil and their toxicity.

#### **4.04.T-03 Effects of the Addition of Organic Amendments on the Adsorption of Atenolol, Ibuprofen and Tetracycline in Two Agricultural Soils**

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The presence of pharmaceuticals in wastewater is an issue of major concern, especially due to its use in agricultural systems, which might lead to an increase of these persistent compounds in the soil-plant system and groundwater. Sustainable practices in agriculture, such as the addition of organic amendments, can benefit not only from the improvement of soil physicochemical and biological properties, but also from the capacity of several organic materials, such as composts or biochars, to adsorb organic contaminants. The aim of this work was to evaluate the potential of four organic amendments to increase the capacity of two agricultural soils to adsorb pharmaceuticals. The two agricultural soils selected for this work, S and B, have similar texture (sandy loam) but different pH (S: 7.44; B: 8.39), OM contents (S: 1.39%; B: 0.77%). Four organic amendments were added to the soils in a 5% ratio (w:w): a hydrochar (HC) and a biochar (BC) from pruning waste, a green waste compost (GWC) and spent mushroom substrate (SMS). The analysis of the chemical composition of the organic materials revealed a high content of aromatic groups in BC, while HC, GWC and SMS showed a more heterogeneous composition and the presence of other functional groups. The three pharmaceuticals studied, Atenolol (AT), Ibuprofen (IB) and Tetracycline (TC), are frequently found in wastewater and the environment and show very different chemical structures and acid dissociation constants (AT:  $pK_a$  9.6; IB:  $pK_a$  5.2; TC:  $pK_{a1}$  3.3,  $pK_{a2}$  7.7 and  $pK_{a3}$  9.7), which greatly affects their mobility in soils. Batch adsorption isotherms were performed with increasing concentrations of ibuprofen (1-20 mg/L), tetracycline and atenolol (50-800 mg/L) for the soils and the soil-amendment mixtures. After 24 h of interaction, pH values were measured in the supernatants and the concentration of the pharmaceuticals was analysed by HPLC-PDA. The data were fitted to the Freundlich adsorption model ( $Q_e = K_f C_e^n$ ). The soil S, with lower pH and higher OM content, showed a higher sorption capacity for AT, IB and TC than soil B. The addition of all the amendments had in general a positive effect on the sorption capacity of both soils for the three compounds, which was more significant for AT. BC and GWC were the amendments that showed a higher potential to improve sorption of pharmaceuticals in agricultural soils in case of multi-contamination events.

#### **4.04.T-04 Harnessing Waterfleas for Water Reclamation**

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Access to clean water is a human right. Yet, more than two billion people worldwide have no access to clean water. Urbanisation, population growth, unsustainable food production and climate change have put unprecedented pressure on water resources systems, leading to global water scarcity. Sustainable use of water resources and water reclamation are urgently needed for people's societal, economic and environmental future. However, water reuse is not safe unless chemical pollutants are removed. Typically, wastewater treatment consists of two main stages that remove pathogens and solids but not persistent pollutants, such as pesticides, pharmaceuticals, and industrial chemicals. Introducing a third stage for the reduction of chemical pollutants typically involves high operational and energy costs, large infrastructure, and can generate toxic by-products (e.g. bromate from

ozonation). Nature-based tertiary treatments are being developed, yet their efficiency is too low for industrial-scale applications and they often demand significant space, infrastructure, and investment, undercutting sustainability objectives.

We have developed a scalable, low-cost, low-carbon, and retrofittable bio-based water bioremediation technology for industrial applications. It uses the waterflea *Daphnia*, a tiny crustacean the size of a grain of rice, as a microscopic vacuum cleaner to non-selectively uptake and retain persistent chemical pollutants and organics from wastewater, enabling water reuse. In a proof of concept study, we benchmarked *Daphnia*'s removal of pharmaceuticals from wastewater against other biological agents, and demonstrated strain-specific removal of chemicals in laboratory settings. Here, we demonstrate the technology at prototype scale (single household) for applications in municipal wastewater treatment. The technology has the potential to maximise the shift to clean growth, enabling water reuse, reducing resource depletion and environmental pollution, and sustaining vital ecosystem services.

#### **4.04.T-05 Bioremediation of Alkaline Spoil Material Using Organic Amendments in the Circular Economy Context**

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Several tunnelling projects have been implementing in Europe and worldwide for easily connecting people in areas with geographical constraints, such as the presence of mountains. For this purpose, tunnel industry produces huge amounts of excavated soil (spoil material) which can be re-used for different purposes, in line with circular economy. The addition of lime can be a practical procedure at a construction site for the chemical stabilization of excavated material making it possible a better managing in the final destination site.

This work reports a nature-based solution, applying different organic amendments (compost, pomace or digestate), for decreasing the highly basic pH (12.5) of the spoil material after adding lime. Microcosm experiments were set-up with spoil material amended with different organic fertilizers and with *Medicago sativa*.

The changes in pH and in the structure and functioning of the microbial community were evaluated in the amended spoil material at 4 months from the start of the experiment. Aerial and root biomass and concentrations of leaf chlorophyll and phenolic compounds were also measured. The microbial community structure was analysed by metagenomic analysis based on the gene for 16S rRNA (NGS); moreover a predictive functional analysis was also performed by data processing with PICRUSt2. Adding the amendments significantly decreased pH values (8.41) and increased microbial abundance and activity. The pomace addition promoted the highest microbial abundance and metabolic activity values and the lowest decrease in pH. However, pomace did not favour plant biomass growth. Compost and digestate showed to be the best solutions for pH decreasing and favouring plant growth. The plant did not have any direct effect on pH lowering, demonstrating in this study the key role of microbial populations in responding efficiently to this environmental stress, mitigating it. In accordance with how mentioned, several genes decoding for membrane proteins (*nuo*, *mrp*) and for indole acetic acid metabolism (*ami*, *nthA*, *ipdC*) involved in pH regulation were found in the amended microbial communities.

#### **4.04.P Bioremediation and Phytoremediation of Aquatic and Terrestrial Contaminated Ecosystems**

##### **4.04.P-Th243 Assisted Phytoremediation: Use of TiO<sub>2</sub> Nanoparticles to Enhance Sunflower Capabilities in Recovering Soil Contaminated by PCBs and HMs**

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Soil pollution is one of the global problems that affected humanity. Recently, Nature-Based Solutions (NBSs) are widely recognized as promising, profitable and sustainable bioremediation strategies to degrade or stabilize organic and inorganic contaminants from the environment. Among these strategies, Plant Assisted BioRemediation (PABR), based on the synergistic action established between root system and soil rhizosphere microorganisms, has been proved to be efficient in restoring historically multi-contaminated soils. In recent years, the use of nanomaterials (particles of 1-100 nm size) in assisting phytoremediation has been studied for recovery soils from pollution. Among these, titanium dioxide (TiO<sub>2</sub>) nanoparticles have been investigated on laboratory-scale to assist phytoremediation of soil contaminated by heavy metals (HMs). The use of TiO<sub>2</sub> nanoparticles for enhancing sunflower capabilities for recovering a soil multi-contaminated from polychlorinated biphenyls (PCBs) and HMs, was assessed in greenhouse microcosms. Sunflower (*Argemone* var) seeds were employed as plant species to favor soil restoration. To set up microcosms, soil was collected from a multi-contaminated area of Southern Italy, located close to Taranto city). Then, it was spiked with TiO<sub>2</sub> nanoparticles, synthesized by IPCF-CNR (Bari). A series of soil microcosms (no planted/ no planted + TiO<sub>2</sub>) were also set up as Control samples. Soil chemical-physical parameters (pH, EC, Available P, Organic C), contaminant analytical determinations and microbiological analyses (microbial abundance and dehydrogenase activity) were carried out at different sampling times (0, 45, 90 days). Moreover, the structure of the main microbiological groups was evaluated by qPCR assays. Pollutants analyses were also carried out on biomass (roots, leaves and stems) samples. The main results obtained in sunflower planted microcosms evidenced that soil PCB concentration reduced at end of the experiment (90 days), showing a re-arrangement of the main congener groups (dioxin-like DXL, marker MRK, non-dioxin-like NO-DXL). HM concentrations in soils didn't vary significantly. HM determinations performed on plant tissues evidenced high accumulation of

Zn and Pb, in leaves and in roots, respectively. The highest values of microbial abundance have been observed in sunflower microcosms spiked with TiO<sub>2</sub>.

#### **4.04.P-Th245 Application of Digestate as a Nature Based Solution for Stemming Antibiotic Resistance Spread in Agroecosystems**

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Cattle manure digestate is a valuable and common resource for obtaining energy (e.g., biomethane), recycling organic waste, reducing odors associated with manure storage and decomposition, and removing harmful pathogens and chemicals. Digestate from the production of biogas is an excellent fertilizer and a good substitute for manure. The use of organic fertilizers, including digestate, has been encouraged at the European level by the introduction of the Fertilizing Product Regulation (EU, 2019/1009). However, in recent years, a great concern arises from antibiotics (ABs) regularly detected in agroecosystems from organic fertilizers. Animal husbandry is one of the most common sources of AB contamination in soils (through direct discharge of urine and feces by medicated animals and application of contaminated manure); the predicted increase in global livestock worldwide in the near future could exacerbate this phenomenon. Recent works have demonstrated how the anaerobic digestion process decreases both the AB (biodegradation) and antibiotic genes (ARGs) concentration of cattle manure used as bioreactor feed. Consequently, the use of digestate as an organic fertilizer can potentially reduce the risk of ABs and ARGs spread in agroecosystems. Results regarding AB biodegradation under anaerobic digestion, in soil amended with digestate and the fate of ABs and ARGs from soil to edible plants (such as lettuce) will be displayed. A significant decrease in AB and ARG transfer from soil to plants in soil amended with digestate, comparing to the same soil amended with manure will be shown. Overall predictive and real functional analyses of ARGs in manure and digestate will support how digestate can be considered a Nature-Based Solution for mitigating the spread of ARGs in agroecosystems.

#### **4.04.P-Th246 Cyanotoxin Degradation in Constructed Wetlands: The Forgotten Degraders**

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Events of harmful cyanobacterial blooms and the subsequent release of cyanotoxins have increased globally in the recent years. This trend is expected to continue as the effects of climate change develop, posing a serious threat to the quality of water resources. Conventional water treatment relies on expensive and energy demanding methods that require specialized infrastructure, restricting their use in isolated and rural areas. As an alternative, constructed wetlands (CWs) have been proposed as a nature-based low-cost solution that can purify water by assimilating nutrients and degrading other contaminants, including cyanotoxins. In fact, CW mesocosms have already been shown to efficiently biodegrade Microcystin-LR (MC-LR). However, the majority of the research in this area only focuses on bacterial degradation, neglecting other microorganisms that may also transform these toxins. Therefore, the aim of this study was to fulfill some of the knowledge gaps on cyanotoxin biodegradation in CWs by uncovering the role of their fungal communities on the removal of MC-LR and cylindrospermopsin (CYN). To do so, different plate culture techniques were used to isolate potential fungal degrader strains from CW mesocosms. The isolates were later identified by Sanger sequencing and finally tested in *in vitro* biodegradation experiments. Moreover, the fungal communities of 28 mesocosms were analyzed by amplicon sequencing. The main results showed that the presence of cyanotoxins explained 23 % of the variation observed in the community composition between the treatments. Even though no biodegradation was observed by the selected fungi, some of the isolated strains belonged to genera harboring MC-LR degrading fungi. Thus, the results from this study, which is one of the few focusing on fungal communities in CWs, should be used as a starting point towards understanding the contribution of fungi to cyanotoxin removal, and their importance in CWs.

#### **4.04.P-Th247 Flumequine Accumulation in *Potamogeton pusillus* Under Hydroponic Conditions**

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Water pollution by veterinary antibiotics (VA) is an increasing worldwide problem, due to intensive animal production practices. Phytoremediation is a low-cost and eco-friendly technology to remove emerging pollutants by the use of plants and associated microorganisms. Nevertheless, operational conditions need to be optimized to achieve efficient removals. *Potamogeton pusillus* is a submerged rooted macrophyte that has proven to be a good accumulator of metals. In this study, we proposed to evaluate the removal of flumequine (VA) by *P. pusillus* and its accumulation in the macrophyte organs. To achieve this goal, we exposed the macrophyte (8 g w.w./L) in hydroponic conditions: Hoagland nutrient solution with or without flumequine at 50 and 500 µg/L, during 14 days. Static experiments were performed in a greenhouse room without temperature/light control (n = 4). Hoagland nutrient solution was renewed every 7 days. Water samples were taken at 0, 2, 5, 7 and 14 days for flumequine analysis by UPLC-MS/MS. At the end of the experiments, macrophytes were carefully washed with distilled water; roots, stem and leaves separated, freeze-dried using liquid nitrogen and homogenized to perform extractions. Flumequine was analysed in each organ by an adapted QuEChERS extraction method, followed by UPLC-MS/MS quantification. Leaves were extracted with ethanol 96% V/V to perform pigments analysis by spectrophotometry. After 14 days, flumequine removal from water was greater at 50 µg/L (62±15 %) compared to the exposure at 500 µg/L (38±12 %). Flumequine accumulation by *P. pusillus* was dose-dependent, with roots showing the higher concentration (7±2 µg/g w.w. and 40±7 µg/g w.w. upon exposure to 50 and 500 µg/L, respectively), followed

by leaves ( $2,3 \pm 0,3 \mu\text{g/g w.w.}$ ;  $13 \pm 2 \mu\text{g/g w.w.}$ ) and stems ( $0,9 \pm 0,4 \mu\text{g/g w.w.}$ ;  $5 \pm 1 \mu\text{g/g w.w.}$ ). Pigments content (chlorophyll-a, chlorophyll-b, pheophytins and carotenoids) decreased after 14 days but ratios of damage (chl-b/chl-a; pheo-a/chl-a) were  $\leq 1$ , indicating no significant damage. Taking into account removal percentages and accumulation of flumequine by *P. pusillus*, this macrophyte could be considered a good candidate for phytoextraction, although further studies under more complex and real conditions are necessary to fully assess this point.

#### 4.04.P-Th248 Adsorption, Leaching and Degradation of Sulfamethoxazole in Amended Soil

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Sulfamethoxazole (SMX) is a wide antibiotic used for human and veterinary medicine. Antibiotics reach agricultural soils via wastewater, sewage sludge, and manure. Their environmental fate is controlled by several phenomena such as soil sorption-desorption and degradation. This work aimed to assess the effects of two organic amendments, biochar (B) and green compost (GC), on the adsorption, leaching, and degradation of SMX in basic agricultural soil. The soil was sampled from a vineyard located in Burgos province (Spain). General soil characteristics: sandy loamy texture, basic pH, low organic matter, and presence of  $\text{CaCO}_3$ . The soil was amended with B or GC at 5%. Adsorption properties of organic amendments and soils were evaluated by adsorption isotherms of SMX. The data were fitted to the Freundlich adsorption model ( $Q_e = K_f C_e^n$ ). The leaching of SMX from soil and B- and GC-amended soils were determined by leaching columns. Finally, the 500g of unamended and B- and GC-amended soils were spiked with SMX and incubated for 42 days. SMX degradation and microbial activity were periodically monitored. Adsorption isotherms reveal the low adsorption capacity of SMX in unamended soil. B and CG showed good adsorption capacity of SMX and the resulting amended soil increased the SMX adsorption capacity of soil. The low SMX adsorption in unamended soil produced quick and intense SMX leaching. B-amended soil reduced SMX leaching. In contrast, GC-amended soil promoted the leaching of SMX. The pH of adsorption isotherms and leachates indicate that the anion of SMX was the major specie in unamended and amended soils. CG amendment enhanced the microbial activity of soil and promoted the degradability of SMX in soil. In contrast, the high adsorption and low biostimulation effect of B in soil reduced the degradation of SMX. The half-life of SMX was 2.6, 6.9 and 11.9 days for GC-amended soil, unamended soil, and B-amended soil, respectively. This work shows the benefits and risks of two organic amendments, B and GC, for the environmental fate of SMX. The different nature of the organic carbon of B and GC was responsible for the different effects on soil. The results highlight the variability of results with respect to the different parameters determined and the need to carefully assess the fate of SMX in amended soils to avoid the accumulation of SMX in soil or copious leaching.

#### 4.04.P-Th249 Cefuroxime Adsorption by Soils Amended with Three Low-cost Bioadsorbents

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The presence of antibiotics in soils is both an environmental and a public health issue, since it favors the proliferation of resistant bacteria, and entails the risk of being introduced in the food chain, which is an important health risk. Cefuroxime is an antibiotic belonging to the  $\beta$ -lactams group that is widely used in the treatment of different diseases, such as bacterial sinusitis, otitis, or tonsillitis. Several studies have found its presence in irrigation waters, rivers and even in food. This antibiotic is excreted by feces and urine, mainly due to the poor absorption in the human intestine, ending up in wastewater. Additionally, the sewage sludge produced in wastewater treatment plants are widely used as fertilizer in agricultural soils, promoting soil pollution.

Adsorption is an effective technique to remove antibiotics from the environment, which favors the growth of studies on the use of low-cost and green bioadsorbents to remove antibiotics from soils. Previous studies demonstrated that cefuroxime adsorption was low in some soils (between 40-70%), and it could be improved adding sorbent materials. For this, in this work three bioadsorbents were added to various soils, to investigate their effect on cefuroxime adsorption in these soils. The bioadsorbents were pine bark, mussel shell and wood ash, which were added to the soils in doses of 12 and 48 t/ha. To carry out this study we used batch experiments, adding solutions with different antibiotic concentrations (0, 2.5, 5, 10, 20, 30, 40, 50  $\mu\text{mol L}^{-1}$ ) at 2 grams of each soil/sorbent mixture.

The results showed that when a bioadsorbent with a basic pH (wood ash or mussel shell) was added, adsorption increased in all cases in comparison with the soil without bioadsorbent. However, when the bioadsorbent with low pH (pine bark) was added, the increase in adsorption was significantly lower than in the case of the basic bioadsorbents, and even for some soils adsorption decreases compared with the soil alone. It shows that cefuroxime adsorption depends on the pH of the adsorbent, conditioning different sorption mechanisms. Desorption was very low for all samples, with the smallest found when the bioadsorbents were added into soils. Both wood ash and mussel shell increase adsorption and decrease desorption for cefuroxime, suggesting that would be suitable bioadsorbents for remediation of soils polluted with that antibiotic.

#### 4.04.P-Th250 Green Technology for Clarithromycin Sorption

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Removal technologies of persistent organic pollutants (POPs) are key in the efforts to reduce the spread and accumulation of these chemical compounds in the environment. The wide variety of POPs and consequently of their properties, represents a challenge in the pursuit of the most adequate removal techniques and is the cause of the highly diverse different approaches that are being

tested, from very high to simpler technologies. Among the POPs, pharmaceutical products, such as antibiotics, are an important target for these removal process since there are widely spread in the environment. One of the paths is spreading on soils through animal slurries or sewage sludge used as fertilizers, with potential detrimental effects in both the environment and the human health. One technique that has gained increasing interest for antibiotic removal is the biosorption, which allows antibiotic retention using different types of bioadsorbents. The problem with some of these bioadsorbents materials is that they may be expensive when made intentionally for that purpose and not sustainable. The aim of our study is to analyse the sorption capacity of different low-cost by-products from the forest and food industry, to test their capacity to effectively adsorb clarithromycin. Batch type experiments were performed were seven different antibiotic concentration (2.5, 5, 10, 20, 30, 40, 50  $\mu\text{mol L}^{-1}$ ) were added to three by-products (wood ash, pine bark and mussel shell). The adsorbed and desorbed antibiotic concentrations were measured by means of HPLC. The results indicate that for the highest concentration of added clarithromycin, the ash showed the highest sorption capacity (92% of the added antibiotic), meanwhile pine bark and mussel shell adsorbed 71% and 67%, respectively. The minimum adsorbed was similar for both ash and pine bark (around 60%), while it was lower for the mussel shell (51%). Regarding clarithromycin desorption, for the highest concentration added, the released concentrations were below the detection limit for the mussel shell, while both ash and pine bark desorbed just 2%. All these by-products (especially wood ash) would be suitable green and sustainable bioadsorbents for the removal of clarithromycin, which could be relevant in the design of green technologies for antibiotics removal.

#### 4.04.P-Th251 Clarithromycin Adsorption in Soils with and without Bioadsorbents

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Many studies have previously shown the presence of antibiotics in soils causing pollution. The increase of antibiotic consumption, followed by the poor absorption in the intestine and unappropriated techniques to remove these antimicrobials from wastewater and sludge in wastewater treatment plants contribute to this problem. After their incorporation in soils through wastewater irrigation and/or sewage sludge spread as fertilizer, antibiotics interact with the solid phase. The subsequent behavior of these antimicrobials depends on the soil characteristics and antibiotic physicochemical properties, and their persistence in the environment will be determined by different process, such as mineralization, degradation, volatilization, transport or adsorption. However, most of the antibiotics that reach the soils remain in the surface due to adsorption reactions. Hence, adsorption processes are of main relevance as regard antibiotics behavior in soils.

Clarithromycin is an antibiotic belonging to the macrolide group, which is characterized by the presence of one macrocyclic lactone ring. Clarithromycin is one of the most consumed antibiotics, and a significant amount of it is excreted by feces and urines after its ingestion. Several studies have found the presence of this antibiotic in rivers, surface and ground waters, in concentrations between 1 and 17  $\mu\text{mol L}^{-1}$ .

Previous works have shown a rather low clarithromycin adsorption capacity for different soils (between 40 and 70%). The aim of this work is to explore the potential improvement of clarithromycin adsorption in soils by adding low-cost sorbents, thus aiding to prevent its entry into the food chain. For this, three bioadsorbents (pine bark, mussel shell and wood ash) were added individually to six soils, in doses of 48 t/ha. To carry out this work we performed batch experiments, adding antibiotic solutions in concentrations of 0, 2.5, 5, 10, 20, 30, 40, 50  $\mu\text{mol L}^{-1}$  at 2 grams of soil without and with bioadsorbent ( added at 48 t/ha). The results showed that in most cases the highest adsorption occurred when soils were added with pine bark, followed by wood ash and mussel shell. In fact, adding mussel shell to the soils decreased adsorption. Finally, desorption values were always less than 10  $\mu\text{mol kg}^{-1}$ , with pine bark associated to the lowest desorption. Overall, pine bark performed as the best option to increase adsorption, while mussel shell showed the lowest efficacy as soil amendment.

#### 4.04.P-Th252 Determining Pharmaceutical Uptake by Wetland Plants to Assess Their Remediation Potential

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Natural treatment systems, such as constructed or natural wetlands, may be a solution for the purification of water from pharmaceutical pollution. Plants are an important part of wetland systems but pharmaceutical uptake in plant tissues is a poorly understood removal mechanism.

The aims of this research are to optimize and validate methodologies for analysis of pharmaceuticals in aerial and below-ground plant tissues in order to evaluate overall uptake. Grinding, extraction, and analysis methods will be optimized for roots, rhizomes, and stems of important wetland plants, *Juncus maritimus* and *Phragmites australis*. After optimizing methodologies, pharmaceutical uptake by the plants when exposed to these compounds in experiments simulating wetland environments will be evaluated. This research will help clarify the role of plants in removing pharmaceuticals from contaminated media so that wetland conditions, including the harvest or selection of plant species, may be optimized.

#### 4.04.P-Th253 Adsorption of Ibuprofen, Tetracycline and Atenolol in Sand, Silt and Clay Fractions of Two Soils

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The presence of pharmaceuticals in agricultural soils is increasing because of the use of wastewater, sewage sludge, and organic fertilizers of animal origin. The physicochemical interaction between the pharmaceuticals and the soil governs the environmental fate of pharmaceuticals and their bioavailability. The aim of this work was to determine the role of the textural fractions (sand,

silt, and clay) of two soils on the adsorption of ibuprofen (IB), tetracycline (TC), and atenolol (AT). These pharmaceuticals were selected because of their different chemical characteristics such as water solubility and  $pK_a$  values. Two agricultural soils (S and B) from Spain with the same textural classification (sandy loam) but different pH (7.44 and 8.44, respectively), organic matter (OM) content (1.39 and 0.77%, respectively) and mineralogy were used. Adsorption isotherms of the three compounds were performed with raw soils and their respective fractions of sand, silt, and clay. After the adsorption process, pH was determined in the suspension and the remaining concentration of pharmaceuticals was analyzed in the supernatant. The data were fitted to the Freundlich adsorption model ( $Q_e = K_f C_e^n$ ). The pH values revealed that the major ionic form was the anion form of IB, the zwitterionic form of TC, and the cationic form of AT in the two soils and in all the fractions. The lowest adsorption took place for IB because of its anionic nature. The adsorption of the zwitterionic form of TC was higher than the cationic form of AT denoting that the cation exchange was not the only adsorption mechanism in soil and soil fractions, including clay. Soil S showed higher adsorption of IB, TC, and AT than soil B. This result can be related to the lower pH, higher OM content, and mineral composition. The adsorption of clay fraction was higher than silt and sand for the two soils and the three pharmaceuticals. The isotherms were of type L and S for IB, type L for TC, and type C and S for AT denoting different adsorption mechanisms in bulk soils and fractions. The mineral composition, OM, and cation exchange capacity were related to the adsorption properties of soil and soil fractions.

#### 4.04.P-Th254 Degradation of Sulfamethoxazole by Immobilized Enzyme-Mediator Systems

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Antibiotics in wastewater have become an issue due to the low removal efficiency of conventional wastewater treatment plants. Sulfamethoxazole (SMX) is a widely used antibiotic in human and veterinary medicine. Laccase can degrade a broad spectrum of organic pollutants. This enzyme catalyzes the degradation of phenolic and non-phenolic compounds. However, industrial application of laccase is limited by its high price, continuous loss of enzyme due to its solubility, and low stability. The immobilization of the enzyme onto solid supports could prevent mentioned drawbacks. The aim of this study is to evaluate the degradation of sulfamethoxazole by a laccase-mediator system using immobilized laccase on two mesoporous materials, activated carbon (AC) and stevensite clay (ST) thermally treated at 500°C, with two different redox mediators, syringaldehyde (SA) and concentrated olive- wastewater (OW). Laccase was immobilized by covalent binding using glutaraldehyde as the linker. Stevensite clay was pretreated by acid wash while no pretreatment was needed for activated carbon. Furthermore, the degradation of this antibiotic using laccase will be compared with other conventional chemical oxidation processes such as Fenton reactions. All the immobilized laccase-mediator systems degraded the antibiotic in a short period of time (5 minutes). AC-SA system achieved a 93% SMX degradation. The others biocatalyst-mediator systems degraded 48% (AC-OW), 50% (ST-SA) and 50% (ST-OW). Furthermore, OW has the potential of becoming a competitive redox mediator capable of achieving, a widely used redox mediator, depending on the carrier used. In advantage, most of the redox mediators used in enzyme catalyzed reactions are costly. OW presents a great advantage due to its waste condition. Fenton reaction degraded a similar amount of antibiotic as the enzyme-mediator systems, showing that the immobilized laccase-mediator systems produce similar SMX degradation than the advanced oxidation processes. This work demonstrates the usefulness of the biocatalyst-mediator systems studied to degrade SMX.

#### 4.04.P-Th255 Absorption of Atenolol and Tetracycline in Different Organic Amendments

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In recent decades, the use of pharmaceutical for human and veterinary purposes has increased considerably. This fact has generated a marked increase in the presence of this type of compounds in wastewater, which is an important problem because some pharmaceuticals are recalcitrant. Consequently, pharmaceuticals usually are in sewage sludge and accidentally enter in agricultural soils as part of fertilization generating a big problem at trophic level, or leaching and contaminating groundwater. The goal of this work is to study different organic amendments as possible adsorbent of these compounds and minimize bioavailability or leaching. A total of four organic amendments were selected for the adsorption study of pharmaceuticals: biochar (B), hydrochar (HYD), compost (COM) and spent mushroom substrate (SMS). Tetracycline (TC) and atenolol (AT) were selected due to their common use and differences in water solubility and  $pK_a$ . A general characterization of the organic amendments was carried out (pH, electrical conductivity, organic matter content, elemental composition...). Furthermore, different spectroscopic techniques were used to obtain more detailed information of the chemical structure: solid-state  $^{13}\text{C}$  nuclear magnetic resonance ( $^{13}\text{C}$ -NMR), infrared (IR). For the absorption analysis, the isotherms were adjusted using Freundlich model. The results showed good absorption in all the organic amendments. In the case of TC, the SMS presented the highest absorption capacity ( $K_f=3025$ ) followed by HYD ( $K_f=1305$ ), COM ( $K_f=1035$ ), and finally B presented the lowest absorption ( $K_f=608$ ). The AT presented lower absorption than TC but for this compound B was the organic amendment with the highest absorption ( $K_f=239$ ), then COM ( $K_f=189$ ), HYD ( $K_f=110$ ) and SMS ( $K_f=20$ ). The different organic carbon nature of these organic amendments could show the different adsorption mechanisms for TC and AT and therefore, the different affinity of organic amendments by each compound. In conclusion, organic amendments can be a potential tool to control pharmaceuticals in the natural environment. Also, the differences in absorption capacity between the organic amendments maybe create new perspectives with the possibility of creating mixtures of various amendments to expand the richness and absorption potential.

#### 4.04.P-Th256 Phytoremediation of Amr Drivers by Aquatic Plants: A Green Barrier to Combat Amr Spread

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There is a growing concern regarding antimicrobial resistance and its spread in the environment. Alongside the detection of chemicals, we also see the development of AMR in the aquatic environment following the release of wastewater and industrial effluents. Various drivers contribute to the spread of antibiotic-resistant bacteria and their antimicrobial genes (ARGs). Antimicrobials, heavy metals, and biocides are classed as AMR drivers and contribute towards a selective pressure resulting in the development of antimicrobial resistance. There is an urgent need to develop tools and processes to remove these drivers from the environment to challenge AMR spread. Aquatic phytoremediation is a recently adopted methodology to remediate contaminated matrices via sequestration and enhanced degradation of chemical pollutants in aquatic environments. An aquatic hyper-accumulator plant, *Lemna minor*, was selected to assess the potential for phytoremediation of common AMR drivers (metals, antibiotics, biocides). For the first time we assess the phytoremediation of AMR drivers in isolation and a combination. Under controlled laboratory exposure two metals (XX), two antibiotics (XX), and two biocides (XX) were used to assess potential combined competition on phytoremediation amongst chemicals of the same class and across different chemical classes. Mitigation measures to combat AMR are needed which account for chemical complexity in the natural environment and the fact that exposure does not occur in isolation to a single driver.

#### 4.04.P-Th257 Reduction of Odors Emitted by Swine Manure Facilities with Bio-foam Cover Technology

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In this pilot-scale study for ammonia (NH<sub>3</sub>) reduction from swine facilities, bio-foam was produced by surfactant solution and ammonia-degrading microbes using automatic foam generation unit and sprayed onto the manure surface. We report that the bio-foam spraying significantly suppresses the release of NH<sub>3</sub> from swine manure (located in the four different real swine manure storage facilities in Yongin city, South Korea) irrespective of the seasonal change. The bio-foam spraying system designed was as effective as the laboratory tests (previous study) in masking and degrading NH<sub>3</sub>, and complete manure coverage was achieved in 40-50 minutes. The applied bio-foam in this study was very dry (Hexagonal, foam quality; 99.0-99.2% and foam density 0.0080 g/mL), medium expansion and stable (half-life; 120 min) with foam bubble density of 25 bubbles/cm<sup>2</sup> (small size bubbles). Furthermore, this study reduced NH<sub>3</sub> significantly higher (90–100 %) compared to the control (without treatment; 25–43 %). Considering this evidence, the bio-foam has two major functions; i.e., bio-foam can act as a physical barrier to prevent early release of malodorous compounds into the atmosphere, and it ultimately degrades malodorous (bad odor gases) by the bacteria, after breaking the foam. In addition, surfactant foam may also serve as an effective medium for bacteria to spread evenly in manure pits.

#### 4.04.P-Th258 Enhanced Rhizoremediation of a Kerosene-Contaminated Soil with a Mixed Bacterial Inoculant Combining Tactic Motility and Biosurfactant Production

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Bioremediation is a main natural-based solution for the clean-up of polluted soils. Pollutant biodegradation is improved by stimulating the activity of the autochthonous microorganisms through the improvement of soil conditions (nutrient content, moisture, pH, etc.) (biostimulation); and/or through the addition of allochthonous microorganisms with specific metabolic traits (bioaugmentation). In heterogeneously polluted soils with organic xenobiotics, it is essential to guarantee the dispersal of the inoculants over the volume of soil and to promote the bioavailability of the pollutants, which will in turn enhance bioremediation efficiencies.

The aim of the present work was to design a rhizoremediation strategy for a kerosene-contaminated soil from a military base (South of Spain), based on the combination of both biostimulation and bioaugmentation in the presence of a model plant (sunflower, *Helianthus annuus*) and at the greenhouse scale (pots with 2 kg of soil). The bioaugmentation inoculant was a mixture of a motile strain (*Pseudomonas putida* G7), which is chemotactic towards sunflower root exudates, and a biosurfactant producing strain (*Bacillus subtilis* DSM10). This inoculant was designed to enhance the dispersion of the biosurfactant producer strain all over the soil volume in pots by chemotactically-mediated comobilization together with the *P. putida* G7 front.

The analysis of the kerosene residue through time showed that its degradation by the autochthonous microbial communities was more efficient in the presence of the plants, and it increased in the presence of both bacterial inoculants. This degradation enhancement was probably due to a pollutant bioavailability improvement provoked by the action of the plants and the surfactants produced by *B. subtilis* DSM10. A decrease in the surface tension of the soil-water extracts of the pots containing *B. subtilis* DSM10 corroborated that the strain was producing biosurfactants. The effect of *B. subtilis* DSM10 on kerosene degradation was only significant in the presence of *P. putida* G7, which is possibly attributable to the dispersal effect provoked by the combination of *P. putida* G7 and the plant.

These results would reflect the importance of achieving a homogenous distribution of the inoculants over the soil volume for efficient soil clean-up, as well as the enhancement of organic pollutant bioavailability, which is one of the mayor constraints of the remediation of soils polluted with organic xenobiotics.



#### 4.04.P-Th259 Influence of Saponin on Pyrene Microbial Degradation in Different Pollutant Carbon Fluxes Relevant for Soil Bioremediation

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The limited biodegradability of slowly desorbing contaminants must be taken into account when developing nature-based solutions (NBS) to soil pollution by polycyclic aromatic hydrocarbons (PAHs). The use of biosurfactants is a well-known strategy for promoting their biodegradation in polluted soils. Although the potential of microbial biosurfactants, such as rhamnolipids, is well known, fitogenic (i.e., plant-produced) surfactants, such as saponin, constitute a realistic alternative, considering sustainability and financial aspects of NBS. This non-ionic biosurfactant, with an excellent performance, low toxicity and wide presence in nature, contains a hydrophilic glycoside backbone and a lipophilic triterpene derivative, contributing to its excellent solubilization capacity for hydrophobic organic contaminants.

The present study seeks to investigate the influence of *Quillaja* saponin biosurfactant on biodegradation of  $^{14}\text{C}$ -pyrene by the PAHs degrader *Mycobacterium gilvum* VM552 under bioavailability restrictions. The experiments were carried out in three different exposure regimes of pyrene generating dissimilar carbon fluxes: supplied as crystals (high carbon flux), supplied by partitioning from loaded polydimethylsiloxane (PDMS- medium) and sorbed to a soil-soot mixture (low), where pyrene was strongly sorbed to soil and soot particles.

The results showed that saponin above and below its critical micelle concentration (0.5 g/L) promoted the biodegradation of pyrene, in different ways depending on the scenario. Significant solubilization and enhanced biodegradation of crystalline pyrene was observed. The enhancement was, however, not so efficient for pyrene-preloaded PDMS and pyrene sorbed into soil-soot mixture. This loss in the efficiency of biosurfactant promoting biodegradation could be explained by the decline in bioavailability and the adhesion of bacteria cells to PDMS surface and soil particles. Our study suggests that saponin can constitute a valid alternative in risk-minimizing strategies at different stages of the bioremediation processes.

#### 4.04.P-Th260 Soil Enzyme Activity in Polluted Soils Treated with Waste-derived Technosols

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Metal mining causes severe pollution problems by releasing potentially hazardous elements (PHEs) into the soil-plant-water system, affecting its ecosystems functions and human health. This study evaluates the effectiveness of Technosols designed to remediate soils affected by the Aznalcóllar mine spill, one of the largest mining accidents in Europe. In particular, the remediation process is assessed by measuring soil enzyme activity (dehydrogenase,  $\beta$ -glucosidase, cellulase, acid phosphatase). Six Technosols (T1-T6) were made by mixing polluted soil (50-60%) and organic/inorganic wastes from mining (iron sludge, marble sludge, and carbonated waste from peatbogs), urban activity (composted sewage sludge, and vermicompost from gardening) and agro-industry (solid olive-mill by-product). The assay included two controls (polluted soil [PS], unpolluted soil [US]), each Technosol [T1-T6] and six treatments of the PS to which the Technosol (T1-T6) was added on top (R1-R6). After 2 months of controlled incubation, soil enzyme activities, soil properties (pH, EC and OC), and soluble and bioavailable PHEs concentrations were analysed. No changes in the different enzyme activities were found in the treatments (R1-R6). Two months were not enough to recover the low biological activity in the PS treated with the different Technosols on top (R1-R6). However, in Technosols (T1-T6) the biological activity in terms of dehydrogenase activity has been stimulated by far compared to the US. Especially in the Technosols containing solid olive-mill by-product (T1 and T4) or composted sewage sludge (T2 and T5) with more than 100  $\mu\text{g TPF g soil}^{-1}$ , while in the US about 27  $\mu\text{g TPF g soil}^{-1}$ . Likewise,  $\beta$ -glucosidase, cellulase, and acid phosphatase activity have increased in Technosols (T1-T6) with respect to PS control, but not in all cases they exceeded those given in US control.

Although no changes in enzyme activity were observed, soil properties in PS treated with Technosols (R1-R6) have generally improved; in particular, pH rose to neutralisation and OC has increased by about 0.5%. Also, the solubility and bioavailability of Cd, Cu and Zn was reduced in R1-R6 soils, although there was an increase in the levels of soluble and bioavailable Sb and bioavailable As (not in soluble). Thus, these Technosols were effective in remediating soils polluted by sulphide mining as they recover soil properties, reduce mobility and bioavailability of some PHEs, and also promote biological activity.

#### 4.04.P-Th261 Bioremediation of Polluted Soils with Petroleum Hydrocarbons by Four Spent Mushroom Substrates

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The remediation of polluted soils with petroleum hydrocarbons is a difficult and expensive task. The bioremediation of polluted soils is an attractive alternative to traditional techniques such as thermal desorption. The objective of this work was to assess the ability of four spent mushroom substrates (SMS) (*Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Agaricus bisporus*) to bioremediate a polluted soil from an oil refinery. This soil was sampled from a refinery in Huelva (Spain). Soil samples were mixed with SMSs at a rate of 10% in weight in reactors of 1L and incubated at 70% MWHC for 40 days at 20°C in the dark. TPHs were extracted by microwaves using a mixture of acetone and n-hexane 1:1 (v/v). Fungal biomass was assessed by the ergosterol content. The ligninolytic activities determined were laccase, Mn-peroxidase and versatile peroxidase. Two enzyme activities were analyzed to assess the soil microbial activity, total hydrolase and dehydrogenase.

The soil was extensively colonized by *A. bisporus* and *P. eryngii*. In contrast, *P. ostreatus* and *L. edodes* showed low colonization. However, no significant differences in ergosterol were found between inoculated microcosms. The SMS of *A. bisporus* significantly enhanced the ligninolytic and microbial activity of the soil with respect to control soil. The biostimulatory effect of the other SMSs was low. The final concentration of TPHs was lower in the four amended soils than in the unamended

soil. The SMS of *A. bisporus* increased 48% the degradation of TPHs with respect to control. The other SMS increased the degradation rate of TPHs 34% (*L. edodes*), 29% (*P. ostreatus*), and 12% (*P. eryngii*). However, the degradation effectivity of hydrocarbons was dependent on the aliphatic or aromatic nature and the long chain of the hydrocarbons and the SMS used. The light aliphatic and aromatic hydrocarbons were more degradable than the heaviest. No significant enhanced degradation was found for the aliphatic hydrocarbons >C35 or aromatic hydrocarbons >EC21. The bioavailability of the different fractions of TPHs was important. The SMS of *A. bisporus* was the most effective SMS to degrade aliphatic and aromatic hydrocarbons because of the ability of the fungi to colonize the soil and the biostimulating potential of this agricultural waste. This work demonstrates the usefulness of SMS, an abundant agricultural waste, to bioremediate polluted soils with TPHs and is the first step of a scaling-up procedure.

#### 4.04.P-Th262 Green Bioremediation with White-Rot Fungi

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Efficient removal techniques are urgently needed to remove emerging contaminants (EC), such as pharmaceuticals from wastewater, in order to protect water resources. A major source of pollution of inland waters are effluent discharges from wastewater treatment plants (WWTPs) because the existing treatment processes do not efficiently remove EC. Bioremediation based on white-rot fungi (mycoremediation) is particularly promising in decontamination because of the nonspecific nature of the ligninolytic enzymatic system, which can degrade a wide range of EC. Direct use of a colonized mushroom substrate is one method of employing oxidative enzymes. From a practical perspective, this would be less resource-intensive than downstream processing and purification of the enzymes. Furthermore, locally produced plant biomass, such as wetland biomass, could be used to propagate the fungal mycelium. Another option may be submerged fermentation for production of mycelium directly in wastewater. Thus, this research focuses on developing novel, circular and resource-efficient mycoremediation strategies. In one study, laccase and manganese peroxidase produced from mushroom substrates colonized by *Pleurotus ostreatus* and other white-rot fungi were evaluated as a novel wastewater treatment method for removal of EC including sulfamethoxazole, erythromycin, azithromycin, fluconazole, trimethoprim, venlafaxine and its metabolite desvenlafaxine, tramadol, lidocaine, metoprolol and caffeine at environmentally relevant concentrations. The selected ECs were treated with suspensions of mushroom substrate with laccase activity ranging between 10-50 U/L.

Overall, removal efficiencies varied significantly between the studied ECs. This demonstrates potential for using mushroom substrate colonized by white-rot fungi for production of lignolytic enzymes which can be useful for removal of ECs from wastewater.

#### 4.04.P-Th263 Biopiles Design for Mycoremediation of Soils Contaminated with Petroleum Hydrocarbons

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The production of mushrooms at an industrial scale generates large amounts of spent mushroom substrate (SMS). The storage and management of this organic waste cause environmental problems ending in landfills or being incinerated. This waste can be used for bioremediation as it contains a valuable microbiota to remediate contaminated soils with organic pollutants. The main objective of this work was to assess the bioremediation of contaminated soil with petroleum hydrocarbons (TPHs) by three SMS (*Pleurotus ostreatus*, *Pleurotus eryngii* and *Agaricus bisporus*) in biopiles. Contaminated soil was obtained from an oil refinery in Huelva (Spain). Two different soil managements were performed, one set of biopiles was built by soil amended with the *A. bisporus* SMS and the other set of biopiles by unamended soil. The biopiles were built by the disposition of a layer of soil, a layer of SMS, and finally, another layer of soil. The three treatments of each set of biopiles corresponded to each one of the three SMS tested. A control treatment without SMS was performed. Biopiles were prepared in 1 L cylindrical and transparent reactors. Moisture was controlled by periodical irrigation. Forced aeration was introduced in the biopiles by a pipette coupled to a pump. The biopiles were incubated for 60 days at 25°C.

Soil moisture was higher for the soils amended with *A. bisporus* SMS than unamended soil, indicating the positive effect of SMS to keep the soil moisture in sandy soil. The enzymatic analysis showed that the highest activities were found in the inferior soil layers. The biopiles built with amended soil with *A. bisporus* SMS and an intermediate layer of *P. eryngii* showed the highest microbial activity. Anyway, all the biopiles with an intermediate layer of SMS reached a final concentration of TPHs significantly lower than the control, reaching up to 65% of TPHs degradation. In general, the inferior layers of soil reached higher TPHs degradation than the upper layers because of the higher moisture levels and microbiota activity. This work demonstrates that the three SMS evaluated enhanced significantly the removal of TPHs, demonstrating the usefulness of this abundant agricultural waste in the remediation of TPHs contaminated soils. The valorization of SMS for this purpose promotes the circular economy and the Green Deal of the European Union reducing the environmental and climate footprint of the food system.

#### 4.04.P-Th264 Bacterial Benz(a)anthracene Catabolic Networks in Soils Are Influenced by HMW-PAHs as Co-substrates

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Polycyclic aromatic hydrocarbons (PAHs) are major environmental pollutants in a number of point source contaminated sites, where they are found embedded in complex mixtures containing different polyaromatic compounds. The application of

bioremediation technologies is often constrained by unpredictable end-point concentrations, enriched in recalcitrant high molecular weight (HMW)-PAHs, and by the formation of generally overlooked PAH transformation products. The aim of this study was to elucidate the microbial populations and potential interactions involved in the biodegradation of benz(a)anthracene (BaA) in a historically creosote-contaminated soil. The combination of DNA-SIP and shotgun metagenomics of <sup>13</sup>C-labeled DNA identified a member of the recently described genus *Immundisolibacter* as the key BaA-degrading population. Analysis of the corresponding metagenome assembled genome (MAG) revealed a highly conserved genetic organization unique within this genus, including novel aromatic ring-hydroxylating dioxygenases. The influence of other HMW-PAHs on BaA degradation was ascertained in soil microcosms spiked with BaA and fluoranthene (FT), pyrene (PY) or chrysene (CHY) in binary mixtures. Co-incubation of PAHs resulted in a major delay in the removal of the less soluble PAHs and an increased formation of benz(a)anthracene-7,12-dione (BaAQ), the ready oxidation product from BaA, which was associated to relevant microbial interactions. Our findings highlight that interacting microbial populations modulate the fate of PAHs when found in mixtures.

#### 4.04.P-Th265 Bacterial Biodegradation Mechanisms to Mitigate the Risk Posed by Polar Transformation Products in PAH-Contaminated Soils

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Genotoxicity of PAH-contaminated soils can eventually increase after bioremediation despite effective PAH removal. This effect has been attributed to the formation and accumulation of polar transformation products, such as oxygenated PAHs (oxy-PAHs). The further biodegradation of these compounds has been described under certain biostimulation conditions; however, the underlying microorganisms and mechanisms remain to be elucidated. With the aim of understanding the mechanisms involved in the biodegradation of oxy-PAHs, we isolated a 9,10-anthraquinone (ANTQ)-degrading bacterial strain and obtained a 7,12-benz(a)anthraquinone (BaAQ)-degrading bacterial consortium. Sand-in-liquid microcosms inoculated with creosote-contaminated soil revealed the existence of a specialized ANTQ-degrading community, and *Sphingobium* sp. AntQ-1 was isolated for its ability to grow on this oxy-PAH. Combining metabolomic, genomic and transcriptomic analysis of strain AntQ-1 we comprehensively reconstructed the ANTQ biodegradation pathway. Specific mechanisms for oxy-PAH degradation were revealed, involving the cleavage of the central ring catalyzed by Baeyer-Villiger monooxygenases (BVMO). Essential genes for the ANTQ biodegradation pathway were encoded in megaplasmid *pANTQ-1*. Abundance of strain AntQ-1 16S rRNA and its BVMO genes in the sand-in-liquid microcosms correlated with maximum ANTQ biodegradation rates, supporting the environmental relevance of this mechanism. In the case of BaAQ, we also identified a specialized BaAQ-degrading subpopulation in the soil using sand-in-liquid microcosms and a BaAQ-degrading microbial consortium was obtained by enrichment in sand-in-liquid cultures with BaAQ as sole carbon source. The integration of data from metabolomic and metagenomic functional gene analyses revealed that the BaAQ metabolic pathway was probably initiated by BVMOs with identical genetic sequences to those encoded in *pANTQ-1*, indicating horizontal gene transfer phenomena. Our results demonstrate the existence of highly specialized microbial communities in contaminated soils responsible for processing oxy-PAHs. Also, they suggest that Baeyer-Villiger oxidations, infrequent during PAH-biodegradation, could be a relevant mechanism for the processing of oxy-PAHs in contaminated sites, thus contributing to mitigate the potential risk of their accumulation.

#### 4.04.P-Th266 Complete Genome Sequence of *Penicillium brevicompactum* (CMG 72), a Polyethylene Microplastics Degrading Strain

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In recent years, there has been an exponential increase in the number of genomic sequences of fungi, which provide information about their genetic, physiological, and ecological diversity. It also shows the great potential of fungi to produce metabolites with pharmaceutical interest.

*Penicillium* species, for example, are very common fungal species and producers of several secondary metabolites and are widely studied and applied in different economic fields. Some of these species are also involved in the decomposition of compounds and have been used in biodegradation studies.

*Penicillium brevicompactum* is an example of a species that can produce an important compound, mycophenolic acid, and its propensity to biodegrade some polymers has also been demonstrated by some authors. In our group, its ability to biodegrade polyethylene microplastics has been studied and demonstrated.

Thus, the annotation of the genome of this fungus will allow the characterization of key genes associated with the biodegradation process, which would be useful in the screening of new microorganisms. Furthermore, the obtained genome would also help to unravel the enzymes and metabolites involved in the biodegradation process, since an annotated genome is essential for all other OMICS studies.

The DNA was extracted from the mycelium cultured in Potato Dextrose Broth, by the guanidinium thiocyanate method. DNA quality was checked with a Nanodrop, and the genome sequence was obtained using Illumina Novaseq. The generated raw sequence was assessed with fastQC and the assembly was done using SPAdes.

After assembly, the genome was analyzed, characterized, and annotated using different web-applications and bioinformatic programs.

The genome shows a total length of 31 099 894 bb, divided into 240 contigs, with a size >=500bp and the largest has a size of 1 505 776 bp. The assembly has a G+C content of 49.22% and 10 907 genes were predicted.

Genes were identified that encode enzymes or proteins already associated with biodegradation of plastics, as well as proteins involved in pathways related to the degradation of chemical compounds.

#### 4.04.P-Th267 Insights into the Phytoremediation of Microplastics from the Aquatic Environment

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Due to global plastic production, microplastic pollution is becoming a serious environmental problem. Therefore, there is enormous interest in methods that can limit microplastics in the environment. Currently, most approaches focus on implementing advanced technologies in existing wastewater treatment plants (e.g., membrane technologies, electrocoagulation), as they represent an important pathway for microplastics to enter the environment. However, microplastics can also enter the environment through other routes or be generated by fragmentation of larger plastic pieces in the environment. In this case, methods for *in situ* removal of microplastics are needed, and one of the promising options is phytoremediation. To date, several studies have documented the attachment of microplastics to the surface of aquatic macrophytes, but quantitative assessment of the amount of adhered microplastics to plant biomass and kinetic studies have not yet been performed. In this context, the aim of our study was to mathematically describe the adhesion of microplastics to plant biomass (duckweed *Lemna minor*) under different environmental conditions with pristine and aged polyethylene microplastics. The results showed that the maximum capacity for microplastics retention was reached after 24 h, with  $2.76 \pm 0.43$  particles/mg and  $0.84 \pm 0.19$  particles/mg being weakly and strongly bioadhered to the plant biomass, respectively. The experimental data were applied to different isothermal models, and the Radke-Prausnitz isotherm best described weak bioadhesion, while strong bioadhesion was best described by the Koble-Corrigan isotherm. Finally, the phytoremediation experiment was performed with pristine and aged particles, and the results showed that pristine particles adhered to a higher extent compared to aged particles. The results indicated that phytoremediation is possible and that its use can be beneficial in removing “fresh” microplastic pollution when microplastics are not yet covered by a biofilm that forms on their surface after aging in freshwaters.

#### 4.04.P-Th268 Enzymes as Bioremediation Strategy Against Microplastics in soil, and its Effects on *L. terrestris*

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Microplastics are increasingly accumulating in agricultural soils worldwide, affecting biodiversity and soil health. For instance, Dr. Esperanza Huerta-Lwanga, previously reported the increased mortality of *Lumbricus terrestris* after exposure to PLA microplastics.

In order to reduce the amount of microplastic in soil, bioremediation strategies could be implemented. Through a collaboration between Maastricht University, RWTH Aachen and Wageningen University, the use of biotechnology was studied as a potential bioremediation strategy against polyester microplastics (PET, PLA, PBAT).

For this first stage of the research, enzymatical tests on polyester microplastics and films were performed *in vitro* at different conditions. The chosen enzymes were leaf-branch compost cutinase (LCC) and FAST-PETase. At the same time, ecotoxicology tests were performed on the effect of different enzyme concentrations on *L. terrestris*.

Our results showed degradation of the polyester substrates with no increased mortality on *L. terrestris* (compared to control tests), proving the potential of this technology in the fight against microplastics.

These preliminary tests could, therefore, set ground for further studies on the enzymatic or microbial degradation of microplastics on soil. Ecotoxicity on other soil organisms should also be analyzed further.

#### 4.04.P-Th269 Comparison of the Remediation Potential of Selected Plant Species for Hexachlorocyclohexane (HCH) Contaminated Sites

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HCHs have been one of the most used pesticides during the last century. Although their use was banned, the remains of their production, processing and application represent the source of contamination. At present, 299 sites are known where these substances have been handled. Phytoremediation using plants and plant-associated microorganisms is one of the applicable methods to remove leaked HCH from the environment.

The four selected plant species (*Juncus effuses*, *Typha latifolia*, *Phragmites australis*, and *Alnus glutinosa*) were repeatedly exposed to either  $\delta$ -HCH or technical HCH (tHCH) at three different concentrations (20; 200 and 1000  $\mu\text{g. L}^{-1}$ ) for three months. HCH isomers were determined in the growing substrate and plant biomass by an established method using GC/MS/MS.

The pesticide removal from the growing substrate is supported by the plants' presence in most cases. The removal intensity depends on the plant species, the concentration of chemicals and the type of treatment ( $\delta$ -HCH or t-HCH). Alder trees provide the highest mean removal efficiency in both kinds of treatments. Followed by *J. effuses* > *T. latifolia* > *P. australis* in the case of the t-HCH. In the case of  $\delta$ -isomer, the order is as follows: *J. effuses* > *P. australis* > *T. latifolia*. The influence of HCH concentration and kind of treatment is evident in all selected plants. The highest removal efficiencies were noticed by the concentration of 200  $\mu\text{g. L}^{-1}$  of  $\delta$ HCH and t-HCH treatment by *J. effuses*, *A. glutinosa* and *P. australis* after correction for the unplanted group. *T. latifolia* showed the highest pesticide removal in the group treated with 1000  $\mu\text{g. L}^{-1}$  of t-HCH and 200  $\mu\text{g. L}^{-1}$  of  $\delta$ HCH. Different trends were observed in the accumulation of HCH in roots and above-ground plant parts. Trans-isomerization was observed in the plant biomass in the  $\delta$ -group. Isomers  $\alpha$  and  $\beta$  were present in the highest concentration, especially in root tissue. The presence of chlorobenzenes as the main degradation product of HCH was observed in all biomass samples. The part of applied pesticides was subject to degradation to unmonitored metabolites, volatilization or complete degradation. This part also depended on the plant species, concentration and the type of treatment.

#### 4.04.P-Th270 Performance of the One and Two Year *Alnus glutinosa* seedlings in Contaminated Soil with Hexachlorocyclohexane - Evaluation its Microbial Abundance in Soil and Rhizosphere

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Hexachlorocyclohexane (HCH) is an persistent organochlorine pesticide consisting eight isomers ( $\alpha$ -HCH to  $\theta$ -HCH). The effect of delta-isomer ( $\delta$ -HCH) and technical hexachlorocyclohexane (t-HCH) on growth, physiological parameters and to microbial and fungal community was studied in different 1 and 2-year-old alder seedlings. Several sets of microcosm pot experiment with exposure to OCP will be performed in order to evaluate growth, differentiation and regeneration of plant tissues together with OCP bioaccumulation and transformation. Changes in the hormonal system of alders were monitored as well. Most of the evaluated characteristics revealed significant differences between the treated and control groups. Furthermore, the presence of plants increased the remediation efficiency by 6.27 - 26.17 %, indicating the plant's role as a remediation accelerator, most likely through released exudates, and a positive effect on the soil microbiome. Higher remediation efficiency for biennial plants without pruning was expected due to higher root biomass, but unexpectedly higher efficiency was observed for annual plants after pruning than without pruning. In the presence of HCH, there was a decrease in Abscissic acid (ABA) and an increase in Jasmonic acid (JA). The intensity of change depended on the plant age. Similarly, Salicylic acid (SA) is increased in annual and decreased in biennial plants.

The amount of the lin genes in chemically treated group exceeds the amount of the lin genes in the control group, thereby proving that bioremediation took place in the rhizosphere of the plant.

The research findings could help us better understand the processes involved in removing pesticides from the soil. The mechanism of in-plant isomerization and the identification of metabolites should be the focus of future research.

#### 4.04.P-Th271 Phytoremediation Potential of *Miscanthus x giganteus* in Organochlorine Pesticide Contaminated Soils Using Polysorbate 80 as a Mobilizing Agent

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There are extensive land areas globally of pesticide-contaminated agricultural soils in need of remediation. In this global environmental challenge, phytoremediation stands as a sustainable, low-cost, biological remediation perspective. *Miscanthus sp.* is a C4 perennial rhizomatous grass and trials to evaluate its potential for phytoremediation of contaminated soils have been reported and are ongoing for sites in Asia and Europe. However, more research on the establishment of a phytoremediation technique for the removal of organochlorine pesticide (OCP) residues of agricultural soils is essential.

Knowing the slow degradation attribution and bioaccumulative nature of the OCPs as well as their low plant availability, the objective of this study was the evaluation of *Miscanthus x giganteus* (MxG) ability, in combination with polysorbate 80 - based microemulsion as a biodegradable mobilising agent, for dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) uptake and remediation potential.

A first six-month greenhouse experiment for the optimization of DDT and HCH uptake was performed. The randomised block design consisted of a control group and three different treatments (T1 – no microemulsion was added, T2 – 0.1 % and T3 – 0.5 % microemulsion concentration). DDT and HCHs contamination concentrations in the treated groups were 765  $\mu\text{g/kg}$  and 105  $\mu\text{g/kg}$  respectively. These concentration levels were achieved by mixing an aged highly contaminated Georgian soil with soil obtained from a local site.

During this experiment, plant phenotypes such as shoot and root development, leaf colour diversity and biomass yield were recorded on weekly basis. These first preliminary results presented unexpected phenotypic significant differences in the plant growth between control and treated groups. Further research to identify possible factors other than OCP contamination, which could explain this outcome, is being conducted. To this end, an ongoing OCP spiked soil experiment will allow us to quantify the plant's fitness and OCP uptake when compared to the aged polluted Georgian soil. Results from chemical analysis on DDT and HCH uptake and translocation in the plant (root-to-shoot ratio) will be presented, and this will address the question of whether the surfactant has boosted the phytoremediation efficiency. Moreover, the fate of OCP accumulated in plants in regard to the transformation products of both DDT and HCH in soil and plant will be studied thoroughly.

#### 4.04.P-Th272 Biodegradation of Organochlorines: Patterns in the Distribution of Biodegradative Functions and the Phylogenetic Origin of Bacteria

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Organochlorines such as chlorinated ethenes (CE) remain to be widely distributed worldwide and, along with their toxic metabolites, still constitute a substantial environmental threat due to actual and/or potential contamination of air, soils or drinking water sources. Although physico-chemical treatment is often necessary at highly polluted sites, natural or substrate-enhanced bacterial degradation of CE and their metabolites in situ is gaining wide acceptance. The spatial distribution of bacteria that metabolize CE (e.g., *Dehalococcoides*, *Dehalobacter*, *Geobacter*, *Dehalogenimonas*, and *Desulfitobacterium*) is far from uniform at contaminated sites. Sensitive methods are required to monitor and guide bioremediation process as well as to enable linkage of this process with specific bacterial taxa. Our major goal is to understand patterns in the distribution of vital biodegradative functions and the phylogenetic origin of bacteria that perform these functions by employing metagenomics and epicPCR. In 2022, we have installed nanofibrous carriers of biomass in drilled wells at two highly polluted sites in Czechia to obtain bacteria with

CE degradation capability. We investigate the contribution of horizontal gene transfer to the distribution of CE degradation genes for reductive dehalogenase by relocating the nanofibrous carriers from contaminated to non-contaminated aquifer.

#### 4.04.P-Th273 Testing Organic Amendment, *Populus nigra* Plantation and Bioinoculation on Mine Polluted Soil Material for Phytoremediation Optimization Purposes

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Mine driven trace elements' pollution entails serious environmental risks and can cause an overall reduction in soil fertility. In the last decades, *in situ* techniques such as phytoremediation have become increasingly important as ways to tackle these negative impacts. The aim of this study was to test the individual and combined effects of different phytoremediation techniques. For this, 64 pots were prepared with mine polluted soils from a mine polluted site from the Phy2SUDE network (SOE4/P5/E1021). In 4 of them the soil was left untreated, while in the remaining 60 pots compost was applied as an organic amendment in a 1:10 (w:w) ratio. No plant thrived in the bare mine soil. Three culture patterns (NP, P and PT: not planted, planted with *Populus nigra* and *P. nigra* planted in co-culture with *Trifolium repens*) with 4 inoculation patterns (NI, BAC, MYC and MIX: no inoculation, plant growth promoting rhizobacteria, mycorrhizae and the previous two mixed) were tested in the amended pots, obtaining a total of 12 different treatments (x5 replicates). After 110 days of plant development, the substrates were collected on the one hand and the plants on the other, separating them into roots, stems and leaves. In the substrates, physicochemical (pH, CEC<sub>e</sub>, total C, total N, Olsen's P, pseudo-total and available elements) and microbiological (metabolic footprint: activity, richness and diversity) parameters were determined. Whereas in plants, observable biometric and phytopathological parameters (stem height, root depth, wilting, chlorosis, pests) were recorded and elemental composition was determined. Multiple 1-ANOVA tests were performed to verify the statistical significance of the differences between groups. The use of the amendment, *P. nigra* plantation and bioinoculation with rhizobacteria turned out to be the best techniques to reduce toxicity and improve soil fertility, as well as to increase the survivability and productivity of the plant itself. Metabolic footprint is markedly different between planted and non-planted soils, and between planted soils inoculated or not with bacteria, which suggests that plant growth regulates the configuration of a microbial community in which the inoculated bacteria thrive comparatively the better. Under the conditions of this study, the use of an organic amendment, a tolerant plant, and plant growth promoting rhizobacteria reduce environmental risk and corrects the infertility of soils impacted by mining activity.

#### 4.04.P-Th274 Influence of Soil Water Content on Energy Crop Summer Rape (*Brassica napus* L.) Phytoremediation Potential

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Soil heavy metal pollution caused by industrialization and anthropogenic activities has become a global and widespread issue. Among the various approaches of heavy metal contaminated sites remediation *in situ*, phytoremediation technologies receive special attention. Soil moisture content variation with ongoing climate change will have an impact on plant biomass production and soil pollutant behavior, and it will affect phytoremediation. The purpose of the study was to determine how soil water content affects the ability of the energy plant summer rape (*B. napus* L.) to phytoremediate Cd-contaminated soil (1-250 mg kg<sup>-1</sup>). Summer rape growth, Cd bioaccumulation and removal efficiency were assessed at optimal, reduced and elevated soil water contents (SWC). *B. napus* demonstrated strong resistance to Cd toxicity as well as the capacity to phytoextract Cd from the soil. While removal effectiveness was determined by rape growth and Cd soil concentrations, Cd accumulation in oilseed rape increased with Cd soil concentration. *B. napus* coped well with low and moderate Cd pollution, whereas high Cd soil pollution had a significant negative impact on plant growth, resulting in low Cd removal efficiency. Plant growth, Cd accumulation, and removal from the soil were influenced by SWC. Oilseed rapes grown in elevated SWC had higher biomass than those grown in reduced SWC, despite the fact that the adverse effect of Cd was more severe at higher SWC. Elevated SWC increased Cd bioaccumulation from soil, whereas reduced SWC resulted in decreased Cd bioaccumulation. The highest Cd removal efficiency is guaranteed by the optimal SWC, whereas a lack of or excess of soil water limits *B. napus* phytoremediation potential and prolongs removal process.

#### 4.04.P-Th275 Development of a Green Electrochemical Degradation Method of ATMP in Water Using Cyclic Voltammetry (CV) on a Graphite Electrode

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The degradation of phosphonates in water is a crucial issue in environmental and analytical chemistry.

Aminotris(methylenephosphonic acid) (ATMP) is an anthropogenic chelating agent containing three C-PO(OH)<sub>2</sub> groups, widely used in industries and is found in surface water in large amounts. It can chelate with metal ions, increasing metal bioavailability and leading to eutrophication. Therefore, aminomethylphosphonic acid (AMPA) is a persistent metabolite of ATMP

biodegradation in nature and a hazardous compound that is responsible for several health risks. Electrochemical degradation (ED) is a promising technique in order to achieve full pollutant degradation prior to the analytical determination. The aim of this study is to develop a green CV method for ATMP degradation in water media. Accordingly, the degradation experiment is carried out in water in the absence of supporting electrolytes and then compared it to a solution containing supporting electrolyte. A pristine graphite electrode (working electrode) vs. Ag/AgCl electrode is employed for CV experiment without any modification steps or added chemicals. The oxidation potential of the ATMP is determined in both electrolytes (0.6 V) and the capacitance of double layer is also measured. ED of ATMP by long-term CV in water showed a significant decrease in the oxidation current along the long-term CV time period of around 60%. This decrease is assumed to be as a result of ATMP concentration drop during the ED experiment. Therefore, the formation of orthophosphate (*o*-PO<sub>4</sub>) indicating a possible ATMP degradation was determined spectroscopically at 820 nm using a molybdenum blue reaction of a formation percentage of 12%. In this study, ATMP is considered as a model substance for phosphonates to investigate the reaction mechanism, degradation products in water media and to develop LC-MS analytical technique simultaneously with *o*-PO<sub>4</sub> determination in order to achieve a mass balance as a future work.

#### 4.04.P-Th276 Carbonaceous Materials Production from Olive Pomace: Possible Valorization Pathways and Application for Remediation of Contaminated Water and Soil

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Portugal is one of the main producers of olives in the world, producing more than 1 million tonnes per year. The main use of the olive is for olive oil production, however, the two-phase system used in its production generates more than 600.000 tons of olive pomace (pasty, anaerobic and phytotoxic), a hard-to-deal residue with a lot of harmful potential to the environment. In this context, the industrial and agricultural sectors have been putting a lot of effort towards the valorization of this residue. One alternative is the production of carbonaceous materials, such as Biochar and Activated Carbon, by the pyrolysis process. These materials can be produced from residues, such as olive pomace, and their application is diverse, from soil remediation, soil conditioner to improve the growth environment of crops, adsorption of heavy metals to the decontamination of polluted waters and ecosystems.

The usual characterization of the carbonaceous materials prepared from residues has a high surface area, and stability, small bulk density, a strong adsorption capacity, weak electrical conductivity, porous structure, and is environmentally friendly and economical. Preliminary studies show that carbonaceous materials from olive pomace have similar characteristics, such as alkaline pH ( $\approx 9.5$ ), high total C (around 68%) and low BET surface area ( $\approx 5 \text{ m}^2 \text{ g}^{-1}$ ), and some methodologies can be employed to improve these properties, as chemical and physical activation.

Nowadays, our group is studying the optimization of new methodologies for the production of carbonaceous materials from the olive pomace, using slow pyrolysis (800°C, 8-12 h) and activations techniques to pre-industrial scale. The produced carbonaceous materials are being tested on contaminant adsorption besides soil application, for water retention and enhancement of soil productivity. Promissory results to simulate the adsorption of contaminated wastewater are being obtained. Some carbonaceous materials were able to adsorb 99% pollutant in 30 minutes. The higher adsorption capacity and faster adsorption kinetics were obtained in the material with a higher surface area, as expected. This early test highlights the carbonaceous materials' potential to remove wastewater pollutants and different ecotoxic tests are carried out to evaluate the potential for freshwater decontamination in mountain rivers.

#### 4.04.P-Th277 Molecular Biology Tools (Mbts) Coupled with Compound-specific Isotope Analysis (Csia) for the Monitoring of a Biological Remediation of an Aquifer Contaminated by Chlorinated Ethanes and Ethenes

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An aquifer contaminated mainly by chlorinated aliphatic hydrocarbons, both chlorinated ethanes and ethenes, was treated by Enhanced Natural Attenuation (ENA), as groundwater remediation intervention. In fact, these compounds may be subject to degradation by specific bacterial populations, whose presence on site had previously been determined through a targeted microbiological/molecular characterization. Considering the large extension of the Area to be treated and the differences in the type of prevailing pollutants, 4 different modules of intervention have been defined according to the chemical composition of the contamination i.e., predominance of one of the two classes of contaminants present in the area, chlorinated ethanes and ethenes. The activities were carried out through the injection within the saturated horizon of 2 different amendments, selected on the basis of the chemical composition of the contamination. Specifically, a solution of lactate and inorganic salts was used in 2 modules, while the remaining 2 modules were treated with a slow-release commercial product.

The injection activity lasted for one year, from spring 2021 until April 2022. The post-operative monitoring phase began in May 2022. In both phases, the monitoring was conducted integrating the chemical data (i.e. trend of the concentration of the target contaminants) with the molecular monitoring of the bacterial species most involved in the anaerobic biodegradation of chlorinated solvents, such as *Geobacter* sp. and *Dehalococcoides* sp., and of the genes corresponding to the key enzymes of the metabolic pathways involved (i.e. *rdhA* involved in the degradation of 1,2-Dichloroethane; *tceA* involved in the degradation step from Tetrachlorethylene to cis-Dichloroethylene; *bvcA* and *vcrA* involved in the degradation step from Vinyl Chloride to Ethylene). The interpretation of the reclamation progress was strengthened by the results obtained from the CSIA (Compound Specific Isotope Analysis) isotope analyses conducted on the <sup>13</sup>C of the contaminants of interest. The overall results obtained will be, in fact, discussed by integrating the three types of data (i.e., chemical, molecular and isotopic) to promptly evaluate the effectiveness

of the interventions in progress and highlight the different trends in the different modules. By combining these methods, a more rapid and precise estimation of the reclamation progress and the implementation of any corrective measures are reached.

#### **4.04.P-Th278 BIOremediation SYStems Exploiting Synergies for Improved Removal of Mixed Pollutants**

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BIOSYSMO is a 48-month EU-action that will develop a computationally-assisted framework for designing and optimizing synergistic biosystems combining the required pathways and traits to achieve the most efficient degradation and sequestration of pollutant mixtures. These biosystems will comprise combinations of bacteria, fungi and plants containing the natural or engineered pathways required for pollutants degradation and identified based on a computationally-assisted analysis. BIOSYSMO will take advantage of the high natural microbial diversity by screening samples from polluted sites and locations affected by diffuse pollution to identify natural microorganisms already present and able to metabolize/transform the target pollutants. The search will be expanded to microorganisms previously identified and characterized by applying data mining tools to genomic and metagenomic data available in public repositories. The construction and optimization of synergistic biosystems will combine approaches based on 1) enhancing plant- microbe (bacteria, fungi) interactions to achieving combinations with improved pollutant uptake and/or degradation; 2) engineering bacteria, for improved degradation and bioaugmentation, and plants (poplar tree), for improved microbial colonization and pollutant uptake; 3) constructing artificial micro-structured consortia into aggregates and biofilms, containing all the required pathways for pollutant removal; and 4) applying bioelectrochemical systems (BES) as stand-alone or in hybrid systems. 5) biostimulating the native microbial communities to exploit natural traits that improve contaminant removal. The different key players will be identified and combined to formulate innovative biosystems with the assistance of genome-scale metabolic (GEM) models for elucidating and simulating the key metabolic pathways. The constructed biosystems will be applied in conventional (phytoremediation, biopile, biostimulation/bioaugmentation) and innovative (BES, hybrid BES-phytoremediation) bioremediation approaches optimized for the treatment of mixtures of pollutants in soil, sediments and waters.

#### **4.05 Characterization, Testing and Assessment of Complex Substances (MCS, UVCBs & MOCS)**

##### **4.05.T-01 UVCB Substances: Characterization, Chemical Representation and Selection of Representatives for (Eco)toxicological Screening Purposes**

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People, animals and the environment can be exposed to various mixtures, including substances of unknown or variable composition, complex reaction products and biological materials (UVCBs). The toxicity potential of the latter has been growing toxicology concern for some years. One of the approaches for assessing the risks UVCBs pose to human and ecological receptors is through understanding their chemical identity. A methodology for UVCB characterization is developed to address this issue. The methodology allows for coding of structural information and generation of individual constituents, using a knowledge for generic substance identifiers, such as names of chemical classes, structural formulas, reaction steps, physicochemical properties, and/or spectral data. In some cases, the number of generated UVCB constituents is too large, which complicates their further (eco)toxicological assessment. Because of that, selecting a manageable number of constituents that represents the UVCB is necessary. Two types of representatives selections are applied depending on the user's goal. The first scheme is an endpoint non-specific selection. To this end, a special algorithm was developed to determine the minimal sample size of randomly selected structures that objectively represent the set of all generated UVCB constituents. The algorithm was further implemented as part of the methodology for UVCB characterization. The second type of selection of a representative sample is endpoint-driven. This is achieved by a novel approach based on hierarchical clustering taking into account different similarity criteria associated with the property under investigation. Finally, one structure is chosen from each obtained sub-group. The selected constituents from all small sets form an endpoint-specific representative sample that could be used for (eco)toxicological screening purposes instead of the whole UVCB. A hierarchical scheme for selection of UVCB constituents for biodegradation modelling is defined based on structural and degradation similarity between the structures. Specific chemical class of a target petroleum UVCB substance is used to analyse the behaviour of the proposed scheme. The scheme showed effective and mechanistically transparent grouping of the chemicals with similar characteristics and properly chosen representatives. Similar approaches for selection of endpoint-specific samples can be developed for other environmental, fate and human health endpoints.

##### **4.05.T-02 Acute Aquatic Toxicity Testing with Isomer-Mixtures of Liquid Organic Hydrogen Carriers**

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Liquid Organic Hydrogen Carriers (LOHC) can contribute to decarbonisation of energy and transportation. Two promising candidates investigated in this work Benzyltoluene (BT) and Dibenzyltoluene (DBT) are already marketed as heat transfer fluids



and are technical isomer-mixtures, which gives these LOHC broad liquidus range and is highly desired. The hydrogenated counterparts (BT-12/DBT-18), are even more complex due to possibility of incomplete hydrogenation. DBT for instance is thus labelled as a UVCB compound by the European Chemicals Agency (ECHA). The difficulties in assessment of these mixtures are additionally exacerbated by hydrophobic nature (log Kow 5-10) and volatility (of BT and DBT). Passive dosing emerged in the last decade as a reliable method to test very hydrophobic compounds in limit as well as concentration-response tests. This was modified here to allow for polymer loading from pure liquid and viscous mixture of LOHC.

Acute *Daphnia magna* immobilisation and *Raphidocelis subcapitata* growth inhibition tests are performed as limit tests. In case of sufficient inhibition, dose-response tests are conducted and EC<sub>50</sub> values are determined. The exposure concentrations are confirmed via GC/MS. We measured log Kow of these compounds and screened their modes of action using toxic ratios approach.

No effect in acute *Raphidocelis subcapitata* and *Daphnia magna* test was observed for BT-H12, DBT and DBT-H18. We speculate that this is due to very high hydrophobicity of the compounds and too short test duration. BT can be classified into acute category I according to GHS aquatic toxicity classification (EC<sub>50</sub> below 1 mg/L). Although only a specific composition of the isomer mixture was tested here, the batch-to-batch differences in H2-lean starting materials are not expected to influence the toxicity to large extent as these compounds seem to baseline toxicants and isomers differ very little in hydrophobicity. The hydrogenated forms of LOHC are more hydrophobic than the starting H2-lean materials. Therefore, incomplete dehydrogenation leading to presence of (partially) hydrogenated compound in the H2-lean compound could theoretically increase (chronic) effects or bioaccumulation potential, as baseline toxicity is the most probable mode-of-action, and the overall water exposure will not significantly change with varying isomer composition.

#### 4.05.T-03 Assessment of Acute and Chronic Ecotoxicological Effects of Aqueous Eluates of Stone Wool Insulation Materials

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Currently, a number of crises, including the global climate crisis and energy supply shortages, affect people worldwide. To limit the anthropogenic climate change to 1.5 °C and to reduce the dependency on fossil fuels like gas for heating purposes, appropriate thermal insulation for buildings especially in temperate regions of the world is needed. Meanwhile, maintaining a high protection of ecosystems makes the ecotoxicological evaluation of all products, including construction materials, necessary. Construction materials often pose a challenge to standardized ecotoxicological testing due to limited water solubility and a complex composition, which is often variable within certain limits.

The test materials investigated in this study were stone wool fibres, which are used in thermal building insulation products and are considered inorganic UVCBs. We assessed the acute and chronic ecotoxicological potential of aqueous eluates of the stone wool fibres. These eluates have been studied with four test species, using standardized laboratory ecotoxicological tests for the following endpoints: acute bioluminescence inhibition (*Aliivibrio fischeri*), acute algae growth inhibition (*Desmodesmus subspicatus*), acute *Daphnia magna* immobilization, chronic *Daphnia magna* reproduction inhibition and chronic nematode growth and reproduction inhibition (*Caenorhabditis elegans*). In parallel, chemical analysis of the eluates was conducted to examine potential leaching of metals and organic substances from the test materials.

The laboratory testing revealed no acute or chronic ecotoxicological effects by the eluates of fibrous stone wool material, while high concentrations of milled test materials lead to some chronic effects on aquatic invertebrates. The chemical analysis showed no leaching of organic carbon from the test materials and a limited elemental leaching, with only two of 18 analysed elements detectable in the eluates. Based on our assessment within this study, aqueous eluates of stone wool fibres should not be considered as chemically hazardous to the aquatic environment. Our study presents a comprehensive test battery, that utilizes aqueous elution and subsequent chemical analysis and ecotoxicological testing with several species of different trophic levels to deepen the current knowledge on the environmental behaviour of an inorganic UVCB construction material.

#### 4.05.T-04 Characterising the Environmental Fate and Behaviour of Diluted Bitumen Within Freshwater Systems

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Extraction and transportation volumes of unconventional bitumen crude oil diluted with naphtha condensates (dilbit, a type of diluted bitumen) will see continued expansion as world reserves of conventional crude oils diminish. However, the environmental fate and behaviour of dilbit is still poorly understood, particularly in freshwater systems. To this end, we conducted spills of dilbit and conventional crude oils (for comparison) in both terrestrial and aquatic model systems to characterise chemical profiles over time and the subsequent embryotoxicity to a North American freshwater indicator species, the fathead minnow. Terrestrial spills of Cold Lake Blend dilbit or Conventional Heavy Blend (CHB) were conducted in large (1 × 0.6 m; H × D) soil columns with artificial precipitation to assess the chemical evolution of oil substituents within infiltrating water of an unsaturated zone.

Leachate water contaminated with dilbit had 2- and 1.6-fold greater concentrations of BTEX and polycyclic aromatic compounds (PACs), respectively. Additionally, tracer tests showed that petroleum constituents of dilbit were transported 6 – 48 % faster in

the infiltrating water. Malformations and expression of *cyp1a* in exposed fish were greatest prior to 34-days post spill. No differences in toxicity were observed between the two oil types, and toxicity could not be correlated to the petroleum contaminants measured (metals, BTEX, PACs, acid extractable organics fraction). Fresh surface water spills were conducted by spilling either Cold Lake Blend dilbit or a Medium Sweet Blend (MSW) within a large meso-scale spill tank. Concentrations of BTEX and PACs were higher in the MSW contaminated water. These higher concentrations correlated with greater sublethal toxic effects of malformations, reduced growth and heart rate in exposed fish. However, significantly greater lethality was observed for up to 2-weeks post-spill for the fish exposed to the water contaminated with dilbit, which could not be directly correlated to the petroleum contaminants analyzed. In conclusion, our studies demonstrated variance in the fate and behaviour of dilbit and conventional oils reflective of their chemical composition (*i.e.*, heavy versus light oil profiles). Further characterisation of the petroleum hydrocarbon classes causing toxic effects is necessary as some endpoints of our studies could not be directly correlated to those compounds most often assessed in oil toxicity literature.

#### 4.05.T-05 Integrating UVCBs and Related Data into Open Chemical Knowledgebases

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Although 20-40 % of chemical registries consist of Substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs), integrating and exchanging information on UVCBs in open chemical knowledgebases is challenging. The integration of UVCBs into high resolution mass spectrometry (HR-MS) based identification workflows is also problematic. Often only a name or numerical identifier is provided in the registry listings, hindering comparison, merging and enumeration or mapping of potential component species either based on expert knowledge or (semi-) automated cheminformatics methods. Improved UVCB handling in major open chemical resources will help support the exchange of information between registries, researchers, and regulators, as well as supporting, *e.g.*, toxicological/environmental assessments and the integration of UVCBs into HR-MS-based workflows. PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), a large open chemical database with over 112M compounds, 298M substances and contributions from over 884 data sources, have recently introduced “concepts” to specifically improve their handling of UVCB-like entities. An initial dataset of ~62K “concepts” was compiled from three large authoritative data sources with a high proportion of UVCBs (FDA GRSR, TSCA and ECHA). Close to 0.5M synonyms (names) were associated with these concepts, which were then used to form the basis for literature mining dictionaries and sets of regular expressions for pattern-based recognition of UVCBs among synonyms. This was validated over several collections. Since UVCBs of variable composition often form (or are expressed as) homologue series, this subset of UVCBs is particularly conducive to automated grouping methods and adaptation to HR-MS workflows. Thus, as a second step, the homologue grouping algorithm OngLai was run over the PubChemLite for Exposomics database (a subset of PubChem with environmentally and toxicologically relevant annotation) and connected to “concepts” using mappings to representative or component structures provided by depositors. Over 163 connections between chemical, homologue series and PubChem Concepts (often many concepts per series) were made; a select few were hand curated and processed so far as proof-of-concept exemplars. This contribution intends to show and discuss potential (and pitfalls) associated with UVCB handling in open resources to support environmental and toxicological use cases.

#### 4.05.P Characterization, Testing and Assessment of Complex Substances (MCS, UVCBs & MOCS)

##### 4.05.P-We299 A Tiered Assessment Framework for Ecological Risk Assessment of Substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs)

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Complex substances such as multi-constituent substances (MCS) and UVCBs usually result from the industrial processing or extraction of natural substances (*e.g.*, essential oils), or from chemical reactions (*e.g.*, Coco Amidopropyl Betaine). Because of the variable and complex nature of source materials, and the potential variability inherent to production processes, these substances can contain many, sometimes uncharacterised, constituents whose concentrations may vary between batches.

The variation of substances between batches can make hazard data difficult to collect and interpret. In addition, testing the whole UVCB substance does not necessarily inform on the behaviour of its constituents, or reveal the presence of risk drivers. As a result, UVCB/MCS risk assessment presents unique challenges to product registrants and regulators alike.

To address these challenges, the UVCB Committee of the Health and Environmental Sciences Institute (a tripartite group including members of the academic, regulatory, and industry sectors) is building an exposure-based tiered approach considering specific types of easily accessible information (namely, Tier 0 information) that may be more suited to the characterization of the complex nature of UVCBs, to integrate it into a fit-for-purpose weight of evidence approach.

The practical use of this framework is highly dependent on the availability and ease of access of Tier 0 characterization and exposure data, as well as the possibility to easily determine whether a substance of interest could be assessed quickly, or whether it might require sophisticated analytical schemes.

We will present this new framework, as well as the progress made on the development of a pilot database of Tier 0 information containing about 100 UVCBs, and the creation of a high-throughput UVCB “complexity” evaluation scheme, and demonstrate how this new framework can help improve or evolve existing approaches to the prioritization and assessment of UVCBs.

#### 4.05.P-We300 Tiered Approaches to Estimating Human Exposure to Mixture Components Via Inhalation and Dermal Absorption

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Assessing human exposure risk from chemical mixture components is challenging, primarily due to the fact that key physico-chemical properties of these components are different in the mixture compared with the pure substance. Therefore, a key area of interest is the rapid estimation of properties that determine the extent of chemical exposure from mixtures. In this work, we have developed and/or tested various approaches to estimating vapour pressure and dermal permeation coefficients, two key properties associated with inhalation and dermal exposure, respectively.

We demonstrate that the vapour pressure of a mixture component can be estimated with increasing accuracy from (1) QSARs based on chemical structure (2) pp-LFERs based on Abraham solvation parameters and (3) COSMOtherm estimates based on activity coefficient estimation derived from quantum mechanics. Dermal permeation coefficients may be likewise estimated for mixture components with increasing accuracy by (1) COSMOperm, a dedicated activity-based model based on quantum mechanics, (2) QSARs based on chemical structure, and (3) log-linear models based on Abraham solvation parameters estimated from chemical structure-based QSARs.

The results demonstrate that inhalation exposure to chemical mixture components can be modelled with acceptable accuracy using exposure models such as RAIDAR-ICE and estimated vapour pressures from the above approaches. It is also demonstrated that dermal exposure is less accurately modelled due to the relatively poor performance of models for dermal permeation coefficient estimation, likely associated with the high uncertainty in the experimental data used to train these models.

#### 4.05.P-We301 Screening Assessment of Endocrine Disruption Properties of a Large Portfolio of Petroleum-Related UVCB Substances

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The European Commission (EC) developed its initial strategy for evaluating endocrine disruptors (EDs) over 20 years ago (COM(1999)706). Currently, the EC is in the process of adopting criteria for identifying EDs under different EU regulations and is considering an amendment to the EU's Classification, Labelling and Packaging (CLP) Regulation. In 2022, the EC proposed new ED hazard categories and criteria for the CLP of substances and mixtures; however, how substances and mixtures will be assigned to these categories and how this might impact other chemical regulations (*e.g.*, REACH) is yet to be determined. Regardless, the potential impact of this amendment to product value chains could be substantial, and there is a critical need for developing technically sound approaches to evaluate ED mechanisms and adverse effects, particularly for highly complex materials like petroleum UVCB substances.

In a first attempt to gather relevant ED information on petroleum UVCB substances, we compiled and evaluated information for a large selection of substances, considering the 2018 ECHA/EFSA guidance for the identification of EDs under the Biocidal Products Regulation or the Plant Protection Products Regulation. The identification of EDs under this guidance requires a rigorous weight-of-evidence analysis of "all available relevant scientific data," a resource-intensive evaluation that is not feasible to implement across a large chemical/product portfolio, like petroleum UVCB substances which may encompass many chemical entities. Therefore, we developed a stepwise, prioritization process: 1) identifying a list of substances of interest; 2) developing a search strategy to efficiently compile *in vitro*, *in silico*, and *in vivo* information related to estrogenic, androgenic, and thyroid pathways from database sources and the scientific literature; 3) employing a "heatmap" approach to visualize the compiled information across sources and pathways; and 4) identifying priorities, uncertainties, and data gaps, and recommending next steps.

Our findings illustrate the challenges associated with compiling ED information using the 2018 ECHA/EFSA guidance, especially for complex chemical portfolios with UVCBs and mixtures. We provide several recommendations and procedures to efficiently compile and evaluate ED information. Our approach can be applied to other chemical/product portfolios to support initial ED screening assessments to identify key data gaps and inform decision-making.

#### 4.05.P-We302 Development of an IATA to Screen for Persistent, Bioaccumulative/Mobile, and Toxic Petroleum UVCB Constituents and Stable Degradation Products

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There is increasing interest in replacing animal tests with computational methods to predict chemical (eco)toxicity. Because of the complexity of toxicity mechanisms, usually several endpoints need to be combined to adequately predict the toxic profile of chemicals. Integrated Approaches to Testing and Assessment (IATA) are based on the integration and translation of the data derived from multiple methods and sources. IATA can incorporate different types of information, such as (eco)toxicity data, computational model predictions, in chemico, in vitro, in vivo, etc. This information can be used to answer different questions in a specific regulatory context.

The current work shows the development of a method to screen persistent (P), bioaccumulative (B) or mobile (M), and toxic (T) constituents generated for petroleum UVCB substances and their predicted degradation products in amounts above 0.1%. For this purpose, an IATA screening the PBMT constituents and their stable degradation products has been developed. The architecture of the PBMT IATA is based on the execution of several layers, each of them providing specific information. The layers are metabolism, persistence, bioaccumulation, mobility and toxicity. Metabolism is the most critical layer as it is responsible for generation of degradation products and identification of those present in amounts above 0.1%. Therefore, the microbial metabolism simulator developed by the Laboratory of Mathematical Chemistry (LMC) has been used to simulate the biodegradation pathways. The PBT/vPvB and PMT/vPvM assessment combines experimental data from the OECD QSAR Toolbox and modelling results from various (Q)SAR models. The assessment criteria have been taken from the regulatory guidelines for PBT/vPvB assessment and the latest draft criteria for PMT/vPvM assessment. Finally, the classification layer provides overall classification for the constituents and their degradation products.

#### 4.05.P-We303 CHANCES2 Project: Testing and in Silico Predicting Acute and Chronic Aquatic Toxicity of Highly Complex Mixtures

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Testing of Unknown or Variable Composition, Complex Reaction Products or Biological Materials (UVCB) poses a regulatory challenge and classical Water Accommodated Fraction (WAF) tests are often criticized as uninterpretable for risk assessment purposes. The CHANCES2 project (Compartmentalized Hazard Assessment for Natural Complex Extracted Substances (Type 2) aims to provide greater certainty in the environmental toxicology assessment of highly Complex Substances. The Type 2 Natural Complex Substances (NCS2) are UVCB of which less than 90% of the composition is known. NCS2s contain also a large fraction of semi-or non-volatiles, resins or waxes which makes them particularly difficult to analyze in the laboratory. Also, lack of information on their composition complicates application of alternative methods such as QSAR. Phase 1 of this project investigated the acute and chronic toxicity of galbanum resinoid to algae and daphnids using a “three block approach”. Galbanum resinoid sample was first divided into 3 blocks (i.e., volatile, non-volatile, non-volatile non-soluble), displaying different chemical characteristics (e.g., monoterpene and sesquiterpene derivatives in volatile block vs. coumarin-sesquiterpene derivatives, ombelliferone and galbanic acid in the non-volatile one). Then, the experimental toxicity of the three blocks to algae and daphnids were assessed. Toxicity of the whole substance was between 10 and 100 mg/L. When fractionated into 3 blocks, the acute toxicity of volatile block was fairly high (between 1-10 mg/L). For the non-volatile block, no acute toxicity was observed. Neither acute nor chronic toxicity was observed for the non-volatile non-soluble block. Also, the results of the toxicity testing and the *in silico* predictions were compared. For the volatile block and the whole substance, the daphnids EC50 were described by the *in silico* predictions, explaining the observed toxicity. Those results evidence that aquatic toxicity of such complex substances can be modeled using appropriate methodology. To date all experimental WAF studies on the blocks have provided quality empirical results in agreement with the *in silico* predictions. Experimental data validate the prediction while the latter support and thermodynamically explain the empirical results from the studies. The project underlines the importance of combining experimental data with accurate *in silico* predictions. Those findings will be confirmed using another NCS2.

#### 4.05.P-We304 Combining Whole UVCB Degradation Tests with Constituent Specific Analysis Can Yield Biotic and Abiotic Degradation Kinetics

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Essential oils are UVCBs (Substances of Unknown or Variable composition, Complex reaction products or Biological materials), which contain volatile and hydrophobic constituents. This makes essential oils “difficult to test substances” that often fall outside the applicability domain of test guidelines, prediction models, and assessment schemes. The environmental testing of UVCBs is normally done by either a whole substance approach or by testing each main constituent separately. The former does not provide constituent specific information, whereas the latter is limited to those constituents that are included in the test.

We have recently proposed an approach that combines whole UVCB biodegradation testing and constituent specific analysis. Fully automated Solid Phase Microextraction (SPME) coupled to GC-MS is here applied directly on gastight test systems. Primary biodegradation kinetics are then determined as constituent depletion based on peak area ratios between biotic test systems and abiotic controls that serve as an experimental and analytical reference. Recently, we observed a decline of several lavender oil constituents in the abiotic controls, which challenged our approach and indicated abiotic degradation. The aim of the study was to separate and quantify biotic and abiotic degradation kinetics of UVCB constituents by the inclusion of an additional freshly spiked analytical reference.

Stream water was sampled in Jydebæk stream (DK) upstream (11.5°C) and downstream (13.5°C) of an effluent discharge point for the local wastewater treatment plant (WWTP). 15 mL stream water was added to 20 mL headspace vials (biotic test systems) and spiked with Lavender oil Barreme type at a test concentration of  $\approx 50 \mu\text{g/L}$ . Spiked ultrapure water was used for abiotic test systems. Biotic and abiotic test systems were incubated at 12°C for 1-28 days. On each day of analysis, triplicate freshly spiked abiotic test systems were prepared and analyzed (SPME GC-MS/MS) along with triplicate incubated biotic and abiotic test systems.

Biodegradation kinetics were determined for most lavender oil constituents and were similar in upstream and downstream water. Linalyl acetate was unstable in the abiotic controls and hydrolyzed with a half-life of 4.4 days. Overall, the inclusion of freshly spiked controls allowed to separate and quantify biotic and abiotic degradation kinetics of the UVCB constituents.

#### **4.05.P-We305 Screening for Persistence Using Whole Petroleum Substance UVCB Biodegradation Testing with Constituent Tracking**

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Petroleum substances (PS) are produced at very high tonnage levels – underlining the need to generate useful and appropriate chemical assessment information. Yet, it has proved extremely challenging to assess the biodegradability of PS due to their UVCB (Unknown, variable, complex reaction product, or biological origin) nature, since the individual constituents may have different partitioning behavior and fates. Typical regulatory biodegradation tests which are optimized for single constituent substances do not function well for PS UVCBs. Specifically, ready biodegradation tests (e.g., OECD 301 series) do not provide information on the biodegradability of individual constituents in PS, and biodegradation simulation tests (e.g., OECD 307-309) do not function well for PS as it is not possible to appropriately radiolabel the numerous individual constituents present in the whole substance. We have previously explored a new method for performing whole PS UVCB biodegradation assessment and the mapping biodegradation half-lives using two-dimensional gas chromatography (GCxGC) with peak tracking (SETAC-Europe 2022, 4.12.P-We213). Over the course of a marine biodegradation test, samples taken at several timepoints were extracted and analyzed by two-dimensional gas chromatography with a flame ionization detector (GCxGC-FID). Thanks to the improved separation of hydrocarbons afforded by the GCxGC technique, it is possible to monitor the biodegradation of individual peaks potentially representing several constituents and then to calculate primary biodegradation half-lives for those peaks. In this effort, about 1100 peaks were tracked, with less than 30 having primary biodegradation half-lives exceeding 40 days. The identity of the constituent(s) in the peaks with the longest half-lives were assessed using GCxGC with a mass spectrometer (MS). The ability to screen the biodegradability of so many constituents was previously only possible with QSAR. This approach can be used to assemble experimental data on groups of related constituents. It also provides data on constituents that would otherwise not have empirical data, as they are not commercially available and difficult to synthesize. The whole substance testing with peak-tracking approach as part of a weight-of-evidence presents a novel answer to the persistence assessment of PS UVCBs.

#### **4.05.P-We306 Elution Model Hydrocarbon Block Method: A Computational Approach to Estimate Hydrocarbon Class and Carbon Number of GCxGC-FID Peaks of a Petroleum Substance UVCB for Environmental Risk Assessment**

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UVCBs (substances of Unknown, Variable composition, Complex reaction products, or of Biological origin), like petroleum substances (PS) pose a challenge for chemical hazard and risk assessment due to the complexity of the substance. One option for simplification of the composition of PS UVCBs, which may contain hundreds to thousands of constituents, is to group those constituents by carbon number and chemical class into what are called hydrocarbon blocks (HCB). Each HCB should be similar in structure and property. The HCB distribution of a PS composition can be determined by allocating the individual peaks from Comprehensive Two-Dimensional Gas Chromatography coupled to a Flame Ionization Detector (GCxGC-FID) to specific HCBs, termed the HCB method (HCBM).

Historically, the HCBM was performed by analytical experts, but this proprietary approach is difficult to replicate. A more computational and reproducible approach, termed the “Elution Model HCBM” is here presented. The elution model, as described in a recent publication (Arey et al., *ES&T*, accepted November 2022), uses an *in silico* approach to simulate the 2-D elution (retention time) pattern of petroleum substances in a GCxGC. To develop the Elution Model HCBM, the elution pattern of a hydrocarbon library of 15,495 individual chemical structures was simulated. The simulated elution patterns were interpreted as probabilistic information about chemical class and carbon number. A 2-D kernel density estimation (KDE) method was then used to determine the regions of the GCxGC chromatogram which approximately contain the constituents of each HCB. This analysis mapped HCB regions onto the GCxGC chromatogram, which then could be used to identify individual GCxGC-FID peaks and also quantify HCBs of real PS samples.

This first version of the Elution Model HCBM is designed to quantify HCBs through the statistical averaging of GCxGC-FID data, but it is not designed to be highly accurate for any individual analyte. The programmed approach is well-defined, and it is amenable to validation, peer-review, and extension. Improvements to the Elution Model HCBM are likely to arise through further testing of the elution model, the associated thermodynamic parameters, the KDE methodology, and the hydrocarbon library, particularly the polynaphthenic, polyaromatic, and naphthenic polyaromatic classes.

#### **4.05.P-We307 Revalidating PetroTox for the Performance of Aquatic Hazard Assessment Using Revised Target Lipid Models**

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Ever since its development in the early 2000s, *PetroTox* has been successfully used to estimate the distribution of complex

petroleum hydrocarbon mixtures among interacting physico-chemical phases and their corresponding toxicity/environmental risk limits. With the emergence of new aquatic toxicity studies with hydrocarbons in recent years, a need to update the core *PetroTox* physico-chemical property and toxicity databases has arisen. A revalidation of *PetroTox* using up-to-date versions of these databases is presented in this work. Recent acute and chronic toxicity data were compiled for approximately three hundred unique hydrocarbons constituents across over 50 aquatic species. The empirical toxicity data were ranked based on reliability, then used to calibrate and evaluate the performance of different organism target lipid models (TLMs) for the prediction of toxicity endpoints. These endpoints are one of the key estimation components of *PetroTox*. The eight TLMs evaluated include partitioning to different types of lipids and lipid surrogates as well as more general target site, rather than lipid, models. The performance of the TLMs fits, and thus prediction of petrochemical toxicity, was evaluated showing the prediction errors among the TLMs were reasonable and very similar to each other, indicating that model selection in this case needed to include criteria other than performance, such as accessibility and practicality. Environmental risk limits (e.g., hazardous concentration for 95% species protection [HC5]) were recalibrated using the selected TLMs. Under a separate related project an updated ecotoxicity database for UVCBs (unknown or variable composition, complex reaction products or of biological materials) was developed. The *PetroTox* model was then revalidated utilizing this updated UVCB database and the newly selected TLMs. This revalidation was an iterative process between the recalibrated TLM and the re-validated *PetroTox* model to balance the variability of partitioning data for mono-constituents and UVCBs in the newly developed ecotoxicity database. This step was crucial to ensure internal consistency between the TLM and *PetroTox* models for regulatory acceptance. This revalidation provides further support to the technical robustness and applicability of *PetroTox* as an environmental modeling tool for the estimation of the toxicity of petroleum products to aquatic organisms.

#### 4.05.P-We308 Evaluating the Impacts of Hydrocarbon Solvent Variability on Environmental Hazard and Persistence

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Hydrocarbon (HC) solvents are a diverse group of petrochemical substances that are generally described as Unknown or Variable composition, Complex reaction products or of Biological materials (UVCBs) and may contain tens of thousands of individual chemical constituents. HC solvents are manufactured from petroleum feedstock following a number of processing steps including distillation, hydrogenation, and hydrosulfurization. However, feedstock and manufacturing processes may differ across regions. As such, hydrocarbon solvent sold under the same trade name may be manufactured in different locations. While solvents sold under a trade name have to meet various company specific specifications (e.g. viscosity and pour point), the difference in manufacturer process and feedstock may result in subtle differences in composition. The goal of this work is to investigate compositional differences between three solvents from different manufacturing locations and compare each using a suite of environmental fate and hazard metrics. Three different solvents were selected (referred to as Aro, Iso, and Exo), each of which is manufactured in the US, EU and Asia (a total of 9 individual substances). Aro's are predominantly aromatics ranging from C11 – C13; Iso's are primarily isoparaffins and naphthenics ranging from C10 – C13, And Exo's are C11 – C15 containing paraffins, isoparaffins and naphthenics. The composition of each was evaluated with two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS), and subsequently modeled for aquatic hazard using *PetroTox*. Additionally, the bioavailable fraction was measured using BE-SPME and ecotoxicological assays were performed using *ceriodaphnia*. Finally, biodegradation testing was conducted using OECD 301F methodology. 2D GC showed that, while the overall carbon distribution of each solvents type was consistent, there were some differences in relative percentage of carbon numbers and chemical class (e.g. relative ratio of isoparaffins to naphthenics). However, despite this *Petrotox*, Be-SPME and *Ceriodaphnia* ecotoxicological data all demonstrate a comparable hazard, and biodegradation rates were also comparable. This study demonstrate that while differences in feedstock and manufacturing process may lead to subtle differences in composition, substances sold under the same trade name in different geographies demonstrate comparable hazard and fate properties.

#### 4.05.P-We309 Photomodification of Low-sulfur-fuel-oils: Investigations of Toxic Effects (POLITE)

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The changes in International Maritime Organization regulations regarding the lower limits of sulfur content in marine fuel oils have initiated the transition to a new generation of low sulfur fuel oils (LSFO). These new generation fuels are diverse, and their physical, chemical, and toxicological properties are less well understood compared to their traditional counterparts. Little is known about the photooxidation capacity of these LSFOs, or the toxicological significance of the range of photo-products they may form. The aim of this project is to improve the understanding of the photomodification potential of LSFOs using both Stage I American lobster larvae, and state-of-the-art analytical capabilities. Trials were conducted with an ultra-low sulfur fuel oil (ULSFO), a conventional heavy crude (CONV, sulfur content ~3.4%), and 12 different LSFOs selected from 49 LSFO products collected from the Australian Maritime Safety Authority (AMSA). Low energy WAFs were prepared and mixed either under the light for 18-hr irradiation or in the dark, and full spectrum light exposures were generated using an Atlas Solar Constant lamp. WAFs were characterized using a fluorometry, a solid phase microextraction coupled to a gas chromatography-flame ionization detection (SPME-GC-FID) instrument was used for biomimetic extraction (BE) measurements, and polycyclic aromatic hydrocarbons and total organic carbon were quantified using USEPA method 3510C/8270C and standard method 5310B. Irradiated and non-irradiated WAFs were prepared with loading rates of 0.01, 0.1, 1, and 10 g/L oil. There was a concentration dependent increase in BE values with increased loading, and irradiation lead to an increase in BE values for both oils. The increase in BE values mirrored the observed toxicity, with the irradiated 10g/L CONV sample having greater effect. WAFs with

the AMSA LSFOs were prepared at 1 g/L under UV light and in the dark and were tested using only 100% strength solution of the WAF. For 11 of the 12 LSFOs tested, observed toxicity in the UV treated WAF was equal or greater than the WAF prepared in the dark. The LSFOs tested were more toxic than the ULSFO and CONV at the 1 g/L loading, and they showed a full range of immobilization response from 10 - 100%. The findings highlight the diversity in LSFO products on the market, and the data generated will be used to develop and validate models, to predict and assess the toxicity of these new generation fuel oils.

#### **4.05.P-We310 Assessing the Toxicity of Oil Sands Process-Affected Water in Early Life Stage Wood frogs (*Lithobates sylvaticus*) using Biomimetic Extraction via Solid-phase Microextraction (BE-SPME)**

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Oil sands process-affected water (OSPW) is a by-product resulting from the extraction process of bitumen in the Canadian oil sands industry and is currently stored in large tailings ponds on site. OSPW is comprised of a mixture of various organic and inorganic constituents. The composition and toxicity of OSPW is influenced by its age, source, and location within tailings ponds. Due to OSPW's complex composition and known acute toxicity to a variety of aquatic life, this experiment is part of a multi-year study aimed at evaluating biomimetic extraction via solid-phase microextraction (BE-SPME) analytical technique for toxicity screening of OSPW. BE-SPME has recently been developed as a cost-effective tool for hazard assessment of the bioavailable organic components in OSPW. We investigated the potential lethal and sub-lethal effects of a range of dilutions of OSPW (80, 40, 20, 10, 5, 2.5 and 1.25%) in wood frogs (*Lithobates sylvaticus*) native to Alberta, Canada from pre-hatching embryonic stages through to metamorphosis. Mortality at 80% of raw OSPW and developmental delays at concentrations of 40% and 80% are evident. As well, low concentrations have significant incidences of deformities (scoliosis and hind limb malformations). In addition to BE-SPME measurements, speciated and total naphthenic acid (NA) concentration analyses of OSPW were conducted. BE-SPME and NA concentration data will be used to derive concentration-effect relationships for OSPW exposure in an environmentally relevant amphibian model. The results will be beneficial for future screening assessment of raw and remediated OSPW in the Canadian Oil Sands.

#### **4.05.P-We311 Sensitivity of Aquatic Organisms to Aromatic and Medicinal Plant Essential Oils and Crude Extracts: A Meta-analytic Approach**

**Sandra Afonso, University of Coimbra, Centre for Functional Ecology, Portugal**

Interest on plant-based products, especially extracts and essential oils, has increased over recent years due to their bioactive and biopesticide properties. Plant products are generally thought to be safe, though plants can produce and accumulate a variety of toxic compounds, with various factors influencing their toxicity. Aquatic organisms can easily be exposed to the toxicological risks of plant-based products, but research exploring the extensive published ecotoxicological data is still limited.

This study aims to conduct a systematic review of published evidence on the acute/short-term and chronic/long-term toxicity of aromatic and medicinal plant (AMP) extracts and essential oils on aquatic organisms. We undertook a systematic literature search using multiple search paths and key words to locate all relevant studies addressing ecotoxicological effects of extracts and essential oils on aquatic organisms that were published in English by November 2022. Studies were screened and inclusion/exclusion criteria allowed the selection of 595 studies in the review. Information on LC50 values was extracted, as well as on variables that might moderate the toxic effects (e.g. plant identity, functional group and origin, plant part, organism identity, functional group). Principal component analysis (PCA) will be carried out to identify the variables most strongly related with toxicity effects. A subset of studies (22) that address ecotoxicological effects on both the standard species *Daphnia magna* (or in alternative *Daphnia* sp.) and other aquatic organisms will be used in a meta-analysis to compare the sensitivity of aquatic organisms to that of *Daphnia* sp.; the effect size is calculated as the response ratio  $R$  ( $LC50_{AquatOrg} / LC50_{Daphnia}$ ). Effect sizes will be summarized using the random-effects model of meta-analysis; overall  $R > 1$  or  $< 1$  indicates lower or higher, respectively, sensitivity in aquatic species than *Daphnia* sp. Potential moderators of toxic effects will be tested using sub-group analyses. Sensitivity analyses will be carried out to assess the robustness of results to analyst decisions and fail-safe numbers will be used to assess robustness of the matrix to publication bias.

The methodological approach used makes available new and relevant information for the development of valid criteria for evaluating the effects of a substantial variety of commercialized AMP essential oils and crude extract on the aquatic environment.

#### **4.05.P-We312 Cytotoxic and Molecular Effects of Soil Extracts from the Agbogbloshie Electronic-Waste Site on the Rainbow Trout RTgill-W1 and Human Caco-2 Cell Lines**

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Electronic-waste (E-waste) sites are notoriously contaminated with complex chemical mixtures thus challenging environmental monitoring, management, and remediation activities. Additionally, there is increasing awareness that traditional whole animal based toxicity tests are resource-intensive, expensive and unethical. Given that E-waste sites are typically situated in low- and middle-income countries that tend to be poorly resourced, there is a need to develop more efficient techniques for application in such settings. The objectives were to: A) characterize cytotoxic effects of soil extracts on the rainbow trout RTgill-W1 and the human Caco-2 cell lines; B) measure gene expression changes in both cell lines, and calculate transcriptomic points of departure (tPODs); and C) compare the cytotoxicity and molecular results across both cell lines to determine if the tPODs are protective of *in vitro* LC50 values. Extracts were prepared from 35 soil samples collected at the Agbogbloshie E-waste site (Accra, Ghana).

Samples were classified as upstream (6), downstream (2), community (3), trade site (8), dump site (13), and burn site (3). The RTgill-W1 and Caco-2 cells were exposed in triplicate to concentrations equivalent to 9.38, 4.69, 2.34, 1.17, 0.59, and 0.29 mg dry weight of extract (eQsed)/ml. Many of the samples from the various site types caused a decrease in RTgill-W1 cell viability % at 9.38 eQsed/ml. For example, obvious cytotoxicity was measured in two upstream and two community samples (6.9 - 66%), five dump site samples (5.2 – 31.4%), and the eight trade site samples (2.2 – 53%). The most cytotoxic site to the RTgill-W1 cells was trade site #8 with an LC50 value of 0.5 eQsed/ml. Preliminary results from the first exposure to the Caco-2 cell line showed that it was less sensitive to the extracts with most sites causing little to no decrease in cell viability; For example, only trade site #8 decreased cell viability by 45% (though this was deemed to be the most cytotoxic sample for both the cell lines). Studies on molecular responses are underway. Differential gene expression upon exposure to these extracts will be measured by ultraplex RNA sequencing technology, from which tPODs will be determined for each site. This work is expected to support ongoing efforts in establishing the use of efficient alternative testing strategies with a focus on developing methods for environmental monitoring activities in contaminated sites at under-resourced locations.

#### 4.05.P-We313 Liquid Organic Hydrogen Carriers (LOHCs) as Novel Carriers of Renewable Energies - Proactive Environmental Hazard Assessment

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Liquid Organic Hydrogen Carriers (LOHCs) are very promising vectors for hydrogen storage and transportation. LOHC systems consist of a pair of H<sub>2</sub>-lean, typically (poly)cyclic aromatic compounds and H<sub>2</sub>-rich form, (poly)cyclic aliphatic compounds. LOHCs have high gravimetric hydrogen storage capacity and can go through multiple hydrogenation/dehydrogenation cycles. Additionally, hydrogen can be stored in LOHC under ambient conditions. The scale of LOHC implementation can reach that of liquid fossil fuels if full replacement shall occur – in this scenario there is a high likelihood of release of LOHCs into the environment. Until now, the LOHC systems based on toluene and benzyltoluene are technically mature but there are some technological hurdles. Thus, it is of high interest to find potential LOHC, which allow for a similar performance but also with similar or better hazard profile. To avoid regrettable substitution (of fossil fuels or one LOHC system by another) a comprehensive hazard profile is crucial before widespread use. This study presents ready biodegradability screening as well as aquatic toxicity assessment of novel LOHC using *Raphidocelis subcapitata*, *Daphnia magna* and *Aliivibrio fischeri*. Due to structural similarity to known endocrine disruptors, this study also reports about estrogenic and androgenic potential. Toxic Ratio approach was used to evaluate whether LOHCs act as baseline toxicants (i.e., toxicity is driven by hydrophobicity). COSMO-RS and COSMOmic were used to predict soil partition coefficient and lipid membrane-water partition coefficient to understand the fate and bioaccumulation of the compounds. Lastly, a comparative hazard assessment was conducted including comparisons between different forms of the same carrier (H<sub>2</sub>-lean vs H<sub>2</sub>-rich forms) and between LOHCs and conventional energy sources (fossil fuels). Our results show that all tested LOHCs seem to be baseline toxicants. Most LOHC compounds are expected to be rather mobile in the soil environment and only H<sub>2</sub>-rich LOHC forms are assumed to have a high bioaccumulation potential. Investigated monocyclic compounds were fully mineralized while bicyclic compounds were only partially degraded in the ready biodegradability test. Overall, tested LOHCs seem to have a similar level of environmental hazard to that of fossil fuel-based system. The research outcome will be of the highest interest to environmental scientists, LOHC developers, regulatory agencies, and the general public.

#### 4.06 Complex Mixtures in Chemical Risk Assessment: Challenges and Opportunities

##### 4.06.P-Th279 Assessment of Developmental Adverse Effects of Complex Real-Life Mixtures Using a Human Induced Pluripotent Stem Cell - Based 3D Bioassay

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Humans are exposed to an unknown number of chemicals from environmental and food sources. Traditionally, hazard identification and toxicological assessment is performed on single chemical level. This approach falls short in describing potential harmful effects of complex mixtures of unknown composition we are exposed to every day. The Green Deal Project “PANORAMIX” aims at determining the effects of chemical mixture extracts from real-life samples using human-relevant bioassays and to identify the mixture drivers. The mixtures investigated are extracts of mixtures of organic chemicals obtained from various pooled samples of water, fish, cow milk, human breast milk and serum (from adult and umbilical cord blood) collected from several European countries. The potential adverse effects of the extracts on development were assessed using the human induced pluripotent stem cells (hiPSCs)based 3D cardio-developmental PluriLum assay. hiPSCs-derived embryoid bodies (EBs) mimicking the human blastocyst were exposed to above extracts during differentiation into cardiomyocytes. Reduction in expression of the cardiac-specific gene *NKX2.5* was quantified by the end of the differentiation regime using a luminescent reporter expressed alongside *NKX2.5*.



Exposure to some extracts of real-life samples negatively impacted the differentiation of EBs within non-cytotoxic levels, when compared to negative control. Cardiac-specific effects were observed in EBs exposed to surface and drinking water, as well as to human cord blood serum.

This work highlights how bioassay analysis of extracts from real-life samples can create value by shedding light on potential harmful exposures that is undetected in our exposome. By using assays such as the PluriLum, it is possible to monitor potential developmental adverse effects of complex chemical mixtures more closely, as well as compare contamination levels across the environment-human-food continuum.

#### **4.06.P-Th284 Combining Polar Organic Chemical Integrative Samplers (POCIS) with Three Standard Bioassays to Evaluate the Impact of Chemical Mixtures in Surface Waters**

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Chemical risk assessment as deployed by the European Water Framework Directive (WFD) might not be sufficient to protect surface waters when considering the contribution of chemical mixture effects on the aquatic environment and subsequently on human health. The proposal of the WFD review has been launched by the Commission in 2022, with the final aim to improve the directive, also on the perspective of the objectives of the European Green Deal and the Circular Economy Action Plan. Main improvements might include sampling schemes with comprehensive exposure times as well as repeated peak concentration exposure time, spanning target analytes, and addressing the impacts of chemical mixtures. The present study is aiming to compare different screening methods, on wastewater treatment plant outflow waters, which combine polar organic chemical integrative samplers (POCIS) with a panel of mode of action (MoA)-specific toxicity bioassays. Two main classes of widely present and highly harmful compounds were investigated: (i) endocrine disrupting compounds using ER $\alpha$ -CALUX and Ligand-Binding Estrogen Receptor Assay (LiBERA) tests and (ii) pesticides using standard algae fluorescence inhibition test measured by pulse-amplitude modulated (PAM) fluorometry. The selected bioassays were tested with both (i) whole field extracts dilutions and (ii) reconstructed spikes test mixture dilutions, prepared with the most representative priority and emerging substances measured in the extracts. Effects measured in the whole extract tests were greater than effects measured in the reconstructed spike tests. Besides, the interference of toxic substances can mask the target MoA, e.g., killing ER $\alpha$ -CALUX cells before the maximum estrogenic agonistic effect is expressed. Practical considerations about the suitability of large-scale implementation of bioassays for monitoring the status of water bodies are provided in terms of required expertise and instrumentation, related costs, time needed to obtain results, limits of detection, etc. Combining passive sampler extracts with standard toxicity tests offers promising perspectives for ecological risk assessment and can be considered complementary to routine chemical analyses.

#### **4.06.P-Th295 How Monitoring Data from the Information Platform for Chemical Monitoring (IPCHEM) Can Help Addressing Mixtures – Two Use Cases**

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The Information Platform for Chemical Monitoring (IPCHEM) is a single access point where EU, national and regional authorities, and researchers can find and share information about where chemicals are found and at which concentrations. IPCHEM allows the user to discover, access and retrieve information on chemical occurrences across different media, such as air, water, soil, food and also in humans. The platform incorporates about 500 million concentration measurements for over 3000 substances. Data originate from regulatory monitoring and research consortia and is continuously integrated following defined quality criteria and harmonisation procedures.

Monitoring data are valuable in the assessment of combined exposures to multiple chemicals, as they allow looking at real-world combinations and exploring trends over time and space. In this poster, we illustrate two mixture studies using data from IPCHEM to illustrate possibilities to the regulatory and scientific community: **Chemical mixtures in the EU population**: in collaboration with the human biomonitoring project HBM4EU, typical mixture compositions at median and higher end exposures (95<sup>th</sup> percentile) were explored based on human biomonitoring data representing internal exposure in the EU adult and children populations. Possible risks were calculated for the single substances and the mixtures using human biomonitoring guidance values. **Spatial distribution of pesticide concentrations at European level**: concentrations of 148 pesticide active substances in stream networks at European level were modelled from their estimated agricultural uses. The screening level model quantified pesticide concentrations in the same order of magnitude of the monitoring data used for benchmarking. The possible mixture risk to aquatic organisms was assessed using a Sum of Toxic Unit approach.

Further examples how monitoring data can support addressing policy and research questions are e.g., direct use in regulatory risk assessments, monitoring compliance and targeting intervention, risk/impact indicators, evaluating the impact of regulatory interventions.

#### **4.06.T-01 Are Pesticide Mixtures at Realistic Environmental Concentrations Noxious to the Freshwater Microalgae *Raphidocelis subcapitata*?**

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Pesticides are intensively used in farming systems worldwide to ensure crop yields, and food safety, with values approaching 350,000 tons of active substances used annually in Europe. Pesticides reach not only to soil even pollute air, and water systems becoming a remarkable risk factor for non-target organisms living there. Albeit the harmful impact of pesticides has been widely documented, most of ecotoxicological studies are focused on single compounds ignoring the real case scenarios where mixtures of pesticides are commonly found in soil and aquatic systems. Developed within the framework of the H2020 SPRINT project (<https://sprint-h2020.eu/>) this study aimed to assess the impact of realistic PPPs mixtures concentrations on the growth rate of the microalgae *Raphidocelis subcapitata*. For this purpose, 11 case studies (CSS) across Europe and Argentina were selected and the concentration of pesticides (>200 active ingredients; a.i.) in water systems nearby agricultural fields was measured in each. Per CSS a mixture of 5 pesticides were defined based on the frequency of occurrence and risk quotient (RQ) calculated for each a.i., totalizing 11 distinct pesticide mixtures. Following the OECD test guideline 201, the microalgae was exposed to each mixture at 4 distinct concentrations: measured environmental concentration (MEC), predicted environmental concentration (PEC), 3\*PEC and 5\*PEC mixtures concentrations.

Of the total of 11 mixtures, 7 have already been tested (PT, SW, IT, CR, CZ, FR, and DE), with significant effects observed in 4 of them (CR, CZ, FR, PT) when exposed to the realistic concentration (MEC). At PEC concentration all mixtures significantly reduced algal growth. Given that all the pesticide mixtures tested contain three herbicides, this may explain the strong inhibition found due to their capacity to interact and modulate the correct photosynthesis of the microalgae. Considering that the MEC tested are generally lower than the lowest NOEC/EC50 values reported for each pesticide, our results reinforce the high risk of pesticide mixtures for aquatic systems, and the need to include more realistic approaches, both considering mixtures and testing environmentally relevant concentrations.

#### **4.06.T-02 Indoor VOCs Levels in retail stores and Employee Personal Exposures in an urban city in Türkiye: Health Risk Assessment, I/O Ratios, and Seasonal Effects**

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This study was carried out in seven various workplaces (bakery, photocopy center, restaurant, hairdresser, dry cleaner, market, and hotel) where different activities were carried out. 43 volatile organic compounds (VOCs) were determined simultaneously in the indoor and outdoor environments of each workplace by passive sampling methodology. Furthermore, to determine the exposure concentrations of the people working in these workplaces, personal sampling was carried out. The sampling studies were repeated in the summer and winter seasons due to the importance of seasonal fluctuations in indoor and outdoor air pollution concentrations. Analyses of the samples were performed by a TD-GC-MS. As a result of the study, it was observed that the specific activities of the workplaces played an essential role in the concentrations of indoor VOCs in the study area. While the highest indoor VOC concentrations were measured at dry cleaners (926.045  $\mu\text{g}/\text{m}^3$ -winter, 1014.1533  $\mu\text{g}/\text{m}^3$ -summer) in both seasons, the lowest indoor VOC concentrations were measured at the hotel (95.25  $\mu\text{g}/\text{m}^3$ -winter) and bakery (85.905  $\mu\text{g}/\text{m}^3$ -summer). All indoor/outdoor ratios (I/O) in workplaces other than the bakery were obtained as > 1. This indicates that most workplaces had higher indoor VOC levels than the outdoor environment due to dominant indoor sources. Besides, for the bakery, photocopy center, restaurant, hairdresser, and hotel, winter indoor concentrations were higher than summer concentrations. The reason for this was that ventilation was reduced during the winter months. In addition to all these outcomes, personal sampling results revealed that most employees were exposed to TVOCs at higher levels than indoor levels. Furthermore, carcinogenic (LTCR) and non-carcinogenic health risks (HQ) due to VOC exposure were estimated using personal sampling results. The total LTCR values of all samples collected from 9 different employees from 7 different workplaces were above the acceptable limit of 1,0E-06 defined by USEPA. The total HQ values were found to be also above the reference value (1) defined by USEPA for the employees working in the photocopy center, restaurant, and hotel reception. The results showed that indoor activities at the workplaces were significant on indoor VOC levels as well as occupational health risks faced by the employees at the workplaces.

#### **4.06.T-03 High-Throughput Effect-Directed Analysis Strategy to Study Thyroid Hormone System Disruptors in WEEE Contaminated Toy Material**

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Plastic products often contain chemical additives (e.g., plasticisers, flame retardants, surfactants...) to improve and/or modify a specific property of the product. However, these additives are also known to be harmful to human health. Although regulation on certain additives exists within the European Union (EU), public concern has been raised about the possible presence of chemical additives in consumer products and plastic children's toys originating from waste electrical and electronic equipment (WEEE). High migration rates of certain additives have been reported in some commercially available toys by simulating the mouthing behavior of children as one of the most significant exposure pathways. The EU restricted the content of some chemicals in toys considering their endocrine disrupting potential. Thyroid hormones play a key role in the development and maintenance of a normal physiological state and a deficiency of such hormones can cause a neurodevelopmental effect. The aim of this study is to elucidate whether chemical mixtures of those polymeric toy materials and their saliva leachates operate as thyroid hormone system disruptors, and to adopt a high-throughput effect directed analysis (EDA) strategy to identify and confirm specific drivers. Saliva leaching experiments were performed in polymeric material from toys commercially available in the EU market. Saliva

and plastic extracts were assessed for their competitive activity with the thyroid hormone in the TTR-binding assay. Samples showing relatively high TTR-binding were later subjected to high-throughput effect-directed analysis (EDA). TTR-binding experiments showed a concentration dependent activity in all analyzed extracts. Components extracted directly from the plastic toys showed the highest TTR-binding capacity. Moreover, saliva leaching extracts from one particular toy appeared to have a non-negligible TTR-binding activity when compared to the others. Two TTR-binding active fractions stood out in all the fractionated plastic extracts. Saliva leaching extracts of one toy showed some minor active fractions as well. This study demonstrates that when using high throughput EDA is a good approach to unravel the potential activity drivers in complex mixtures.

#### 4.06.T-04 Combining Algal Growth Inhibition, Flow Cytometry and Computational Approaches for Chemical Mixture Ecotoxicity Assessment

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Risk assessment of contaminants present in freshwaters is usually carried out according to 'single' compound approaches, assuming that ecosystems are exposed to only one chemical at the same time. Chemical substances are therefore evaluated one by one, independently of possible co-exposures with other compounds. However, ecosystems are generally exposed to chemical mixtures, leading to unknown ecotoxicological effects. In this context, the component-based approach (CBA) can be used when the components of a mixture are clearly defined and consists in predicting the ecotoxicological effects of a combination of substances based on the individual toxicity of each component present in the mixture by using models of joint action. Using the CBA, the present work aimed to assess the joint toxicity of several disinfection by-products (DBPs) in binary mixtures (with three different concentration ratios) on the microalgae *Raphidocelis subcapitata* through the evaluation of the microalgal population growth inhibition coupled with a flow cytometry (FCM) approach for subcellular effects. FCM is able to assess effects on the physiology and morphology of algal cells and thus provide information about the potential underlying causes of toxicity (mode of action) and interaction of the substances in a mixture. Determination of the interaction (additivity/synergism/antagonism) was achieved by comparison with a model of joint action.

We discuss here three binary combinations of substances, monochloroacetic acid (MCAA)-trichloroacetic acid (TCAA), MCAA-Bromochloroacetic acid (BCAA) and TCAA-BCAA. Additivity effects were observed for both MCAA-TCAA and TCAA-BCAA on *R. subcapitata*. In the case of MCAA-BCAA mixtures, only one mixture showed an additivity response, the other two resulting in significant synergistic effects on the growth inhibition endpoint. Additivity observed in most mixtures could be explained by the combined approach of algal growth inhibition and FCM. Indeed, similar effects were observed for each single substance on the physiological and morphological parameters tested, potentially meaning that the substances share the same molecular target in the microalgae in accordance to the CA model. However, further work on the MCAA-BCAA mixture will be needed to confirm and explain the observed synergetic effects. Finally, testing different concentration ratios by combination of DBPs allowed us to generalize the interaction of a specific mixture.

#### 4.06.P Complex Mixtures in Chemical Risk Assessment: Challenges and Opportunities

##### 4.06.P-Th281 Endocrine Disrupting Potential of Real-life Chemical Mixtures from Pooled European Environmental, Food and Human Samples

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Humans are not exposed to single substances, but rather to multiple chemicals simultaneously. Therefore, it is crucial to evaluate the impact of chemical mixtures on human health and encourage mixture risk assessment. Our aim was to evaluate the endocrine disrupting potential of real-life chemical mixtures extracted from pooled environmental, food and human samples.

Two OECD validated reporter gene assays, namely human estrogen receptor (hER; TG455) and human androgen receptor (hAR; TG458) transactivation assays, were used. Cytotoxicity was measured and concentrations inducing more than 10% of cell death were excluded. Chemicals were extracted from pooled samples collected from various European countries to get as close as possible to an average European chemical mixture in each matrix. Effects are reported as relative to maximum effect of positive control.

Wastewater (WW) and surface water (SW) showed hAR agonistic activity below a REF of 2 L<sub>water</sub>/L<sub>bioassay</sub>. Wild (FFW) and lean fatty fish (LFW) exhibited 2-fold higher hAR antagonistic activity compared to aquaculture fatty fish (FFA). Organic (OCM) and conventional cow milk (CCM), as well as human breast milk (HBM), exhibited hAR antagonistic activity below a REF of 2 mL<sub>milk</sub>/mL<sub>bioassay</sub>. The highest antiandrogenic potential was found in serum from adult females and in cord blood (IC<sub>10</sub> values below 0.2 mL<sub>serum</sub>/mL<sub>bioassay</sub>).

The ranking of estrogenic potency of water samples was as follows: WW > Effluent > SW > Bottled water > Drinking water. FFW and LFW, but not FFA, also exhibited estrogenic activity. Cow milk samples showed ER agonistic effects as well, whereas no effect was observed in HBM. Both adult and cord blood serum samples showed significant ER agonist activity (EC<sub>10</sub> values below 0.3 mL<sub>serum</sub>/mL<sub>bioassay</sub>).

Our work emphasizes the potential of *in vitro* reporter gene assays as useful tools to assess endocrine disrupting effects of real-life chemical mixtures. Our results indicate that endocrine disrupting activity appears in samples across the environment-food-human continuum. Future studies include the identification of the chemical mixture drivers in these samples by effect-directed analysis.

#### 4.06.P-Th282 Food Packaging – Effects of Recyclable Plastic

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There is an increasing demand from society towards using recyclable materials in food packages. However, there are concerns that hazardous chemicals existing in the recyclable material may migrate into food and pose a health hazard for humans. To gain more knowledge about the potential hazards, effect-based methods, were used to understand the effects of known, unknown and mixtures of chemicals. The panel of effect-based methods included oxidative stress, xenobiotic metabolism and endocrine system effects in recycled plastic materials intended to be used in food packages. In total, migration of eight recyclable plastic materials was conducted using ethanol according to the European legislation, which then were tested at concentrations of 0.006 - 0.050 cm<sup>2</sup>/mL. No effects were seen for oxidative stress or antagonistic estrogen response, while only one material induced xenobiotic metabolism. The plastic materials tested consisted of polyethylene and low-density polyethylene/linear low-density polyethylene, of which the former showed the effect. To summarize, an effect-based approach enables hazard assessment of chemicals within food packages, which is a valuable tool for ensuring safe use of these types of packing materials.

#### 4.06.P-Th283 Risk Assessment of Mixtures of Antiandrogenic Chemicals Based on In Vitro Hazard And Human Biomonitoring Data

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In our everyday life, humans are exposed to numerous chemicals stemming from environmental sources, food, and consumer goods. Multiple component-based mixture studies have shown that chemical mixtures can induce effects greater than those induced by the single chemicals on many human health endpoints, including male reproductive health disorders, for which human androgen receptor (hAR) antagonism emerges as a key molecular initiating event. Mixture risk assessment (MRA) is commonly performed using *in vivo* rodent data and human exposure data from food intake or environmental exposure. Due to the lack of *in vivo* data for many chemicals, the questionable relevance of rodent data, and the risk of lacking the most relevant exposure sources, there is a need to explore alternative MRA approaches. In this case study we explored a methodology for MRA of antiandrogenic chemicals based exclusively on human-derived hazard and exposure data.

A comprehensive literature search was conducted to retrieve hazard and exposure data of 231 compounds previously reported as hAR antagonists. The ratio of human blood levels by hazard values (IC<sub>10</sub> values for hAR antagonism), were used for deriving Risk Quotients (RQs) for each chemical. For a rough measure of cumulative risk, the Hazard Index (HI) was calculated by summing up the RQs estimated for each component of the mixture.

From the total of 231 substances, reliable data was retrieved for 61 chemicals. No chemical showed an individual RQ above 1. The HIs for the entire mixture were 0.32 and 0.98 based on mean and maximum exposure levels, respectively. The mixture risk drivers, i.e. the compounds that contributed the most to the HIs, included polychlorinated biphenyls (PCBs), phthalates, the UV filter benzophenone-3, perfluorooctanesulfonate (PFOS), methylparaben, some pesticides, and a polycyclic aromatic hydrocarbon metabolite.

The aim of the study was to assess whether combined exposure to antiandrogenic chemicals with direct action on the androgen receptor *in vitro* may have an impact on human health. Our approach has pinpointed compounds that contribute markedly to the HI, such as PCBs, phthalates, benzophenone-3 and PFOS. Moreover, the calculated HIs provide a basis for a better understanding of the extent to which the combined chemical load will potentially exhibit adverse antiandrogenic effects in humans.

#### 4.06.P-Th284 Combining Polar Organic Chemical Integrative Samplers (POCIS) with Three Standard Bioassays to Evaluate the Impact of Chemical Mixtures in Surface Waters

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Chemical risk assessment as deployed by the European Water Framework Directive (WFD) might not be sufficient to protect surface waters when considering the contribution of chemical mixture effects on the aquatic environment and subsequently on human health. The proposal of the WFD review has been launched by the Commission in 2022, with the final aim to improve the directive, also on the perspective of the objectives of the European Green Deal and the Circular Economy Action Plan. Main improvements might include sampling schemes with comprehensive exposure times as well as repeated peak concentration exposure time, spanning target analytes, and addressing the impacts of chemical mixtures. The present study is aiming to compare different screening methods, on wastewater treatment plant outflow waters, which combine polar organic chemical integrative samplers (POCIS) with a panel of mode of action (MoA)-specific toxicity bioassays. Two main classes of widely present and highly harmful compounds were investigated: (i) endocrine disrupting compounds using ER $\alpha$ -CALUX and Ligand-Binding Estrogen Receptor Assay (LiBERA) tests and (ii) pesticides using standard algae fluorescence inhibition test measured by pulse-amplitude modulated (PAM) fluorometry. The selected bioassays were tested with both (i) whole field extracts dilutions and (ii)

reconstructed spikes test mixture dilutions, prepared with the most representative priority and emerging substances measured in the extracts. Effects measured in the whole extract tests were greater than effects measured in the reconstructed spike tests. Besides, the interference of toxic substances can mask the target MoA, e.g., killing ER $\alpha$ -CALUX cells before the maximum estrogenic agonistic effect is expressed. Practical considerations about the suitability of large-scale implementation of bioassays for monitoring the status of water bodies are provided in terms of required expertise and instrumentation, related costs, time needed to obtain results, limits of detection, etc. Combining passive sampler extracts with standard toxicity tests offers promising perspectives for ecological risk assessment and can be considered complementary to routine chemical analyses.

#### 4.06.P-Th285 Cost-Effective and Effect-based Evaluation of the Wastewater Quality Using a Battery of In Vitro Bioassays

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Wastewater treatment plants (WWTPs) represent a key point source of contaminants of emerging concern (CECs) into the environment. The continuous monitoring of performance of the WWTPs to avoid influx of contaminants to the ecosystems is therefore important. In this study, a battery of *in vitro* bioassays covering a wide spectrum of effect endpoints is proposed for wastewater monitoring (estrogenic, anti-androgenic, glucocorticoid, PPAR $\gamma$  receptor, PAHs, oxidative stress, and pregnane X receptor activities). The bioassays can act as an early warning system by addressing the mixture toxicity (combined adverse effect) of multiple contaminants. The battery was applied to 11 WWTP effluent samples from the Danube River Basin (DRB), Selection of WWTP was based on the dominant removal technology of the country and the size of population served by the plants. The results indicated that the treatment technologies adopted by the studied WWTPs are unable to remove efficiently certain groups of CECs that cause specific effects including estrogenicity, PAH activity, xenobiotic metabolism and oxidative stress. The measured signals were benchmarked against the effect-based trigger values (EBTs). A mitigation plan was proposed to the WWTP operators based on the extent of exceedance of EBTs. This study demonstrates that the effect-based monitoring battery is a complimentary tool to the chemical analysis approach for quality control of wastewater. A regular application of such time- and cost-effective bioanalytical tools in the WWTPs of DRB is proposed to provide a 'safety net' for the aquatic ecosystems.

#### 4.06.P-Th286 Effects of Bisphenols and Their Mixtures on The Estrogen Receptor Transactivation in Cultured Hera-hela-9903 Cells

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As the use of household chemical products increases, the risk of chemical mixtures also increases and a more precise evaluation of the mixture toxicity is required, but studies on mixture toxicity are not yet sufficient. In this study, the transcriptional activity of estrogen receptor (ER) was measured based on the OECD Test Guideline 455 for the 9 bisphenols including bisphenol A, bisphenol AF, bisphenol B, bisphenol C, bisphenol E, bisphenol P, BPS-MPE, bisphenol Z, and 2,4-BPS. As results, bisphenol A, bisphenol AF, bisphenol Z, bisphenol B, bisphenol C, and bisphenol E showed agonistic activity while BPS-MPE, 2,4-BPS, and bisphenol P showed antagonistic activity. For the mixtures toxicity of bisphenols, some of agonists and antagonists were mixed for binary and ternary combination : agonist/agonist, antagonist/antagonist, agonist/antagonist. The ER transcription activity for the mixtures were measured according to the test guideline and the results were compared to the predicted values obtained by full logistic model (FLM). As results, the parameters (Max, Min, EC50, and Hill slope) obtained from the concentration-response curves of the measured and the predicted were very similar. This result suggests that POD (point of departure) value of the bisphenols mixture can be obtained from *in silico* model without testing and it may be one of tools for the next generation risk assessment (NGRA).

#### 4.06.P-Th287 Composition-Specific Th17 Responses Induced by Real-world Air Pollution and Amelioration by Vitamin D

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Individuals with chronic respiratory disease (e.g., asthma, COPD) are particularly susceptible to poor air quality, whilst serum vitamin D (VitD) is inversely associated with disease exacerbations. Ambient particulate matter (PM) composition is highly complex containing a significant organic component, including aryl hydrocarbon receptor (AhR) ligands. The AhR is an environmental sensor required for Th17 maintenance, though different ligands may have contrasting immunoregulatory roles. We hypothesised that real-world particulate matter (PM) contributes to Th17-type responses linked to severe respiratory disease by perturbing AhR signalling within DCs and CD4 T cells, that PM effects are composition-specific, and that VitD supplementation is protective.

**Methods:** Human monocyte-derived dendritic cells (DC) were stimulated with ambient real-world PM (10 $\mu$ g/mL) from London (PM<sub>2.5</sub>, PM<sub>10</sub>) and Thailand (urban/rural PM<sub>2.5</sub>), or an endogenous AhR ligand (FICZ, 200nM), *in vitro* for 20 hours. Autologous memory CD4<sup>+</sup> T-cells (Tm) were co-cultured with PM-exposed DC, with or without a selective AhR-antagonist (CH223191, 10 $\mu$ M), or 1,25-dihydroxyvitamin D (100nM) for 7 days. DC maturation and T-cell phenotype was assessed by flow cytometry and RT-qPCR.

London PM<sub>10</sub> (p<0.0001) and PM<sub>2.5</sub> (p<0.001), but Thai PM<sub>2.5</sub> from Chiang Mai and Bangkok did not increased expression of DC maturation markers such as HLA-DR. Tm proliferation, Th17 frequency, d AhR expression, which was not restricted to the Th17 compartment, were increased by co-culture with both London and urban Thai PM pre-treated DCs. AhR antagonism in coculture reduced Tm proliferation (approx. 50%), IL-17A+ frequency and AhR target gene expression (cytochrome p450s, IL-22). FICZ-treatment and AhR antagonism in DCs had no effect upon DC/Tm phenotype. VitD treatment significantly reduced DC maturation, subsequent CD4 proliferation and Th17 responses.

PM pre-treated DC induce both T cell expansion and Th17-associated cytokine production, and these effects appear PM-source dependent. The AhR plays a role in T-cell expansion, but is not obviously linked to PM-induced effects on DC. Future organic compositional analyses of real-world PM samples linked to functional data may provide insight into PM driven signalling pathways. VitD significantly reduced PM-induced inflammatory responses, placing it as a promising therapeutic to mitigate against PM-induced inflammation.

#### 4.06.P-Th288 In Vitro Comparative Metabolism of Chemicals that are Nominally a Gas

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The requirement to perform *in vitro* comparative metabolism studies for pesticide active substances related to human health is defined by Commission Regulation (EU) No 283/2013, setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and the Council concerning the placing of plant protection products on the market. *In Vitro* Comparative Metabolism (IVCM) is required to assess the potential toxicity of agrochemicals to humans; comparison is typically made between human microsomes, S9 fractions, or hepatocytes and the equivalent test system from the toxicological assessment species (rat, dog, mouse, rabbit).

The EFSA Plant Protection Products and their Residues (PPR Panel) working group has been focussing on how best to do this, and a scientific opinion was published in 2021. However, this does not address how to deal with chemicals that are nominally a gas, yet an IVCM study is requested by the regulatory authorities.

This presentation discusses the challenges of performing an *in vitro* comparative metabolism study on a <sup>14</sup>C-labelled water-soluble gas in relation to recovery, metabolism, and analytical challenges.

#### 4.06.P-Th289 Induction of CYP1A1 by Benzo[a]pyrene and Phenanthrene in zebrafish hepatocytes

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Polycyclic aromatic hydrocarbons (PAHs) are persistent contaminants ubiquitously present in the environment. Anthropogenic activities, namely fossil fuel combustion are the main sources of these compounds.

Once in the aquatic environment, PAHs are hardly degraded owing to their physical-chemical properties and therefore persist and accumulate in sediment beds being available to cause harm to several organisms.

Many PAH compounds are procarcinogenic, thus requiring hepatic biotransformation to form reactive metabolites and exert toxicity. Cytochrome P450 monooxygenase system is responsible for phase I transformation in which those reactive metabolites are formed. The quantification of CYP1A1 subfamily members is a common and widely used biomarker of polycyclic aromatic hydrocarbon exposure.

One of the most important features of PAHs is that they always appear as complex mixtures but, nonetheless, research has focused mainly on the effects of single compounds. It was already proved that mixtures may trigger totally different toxicological effects on organisms since interactions between compounds may result in an unpredictable outcome compared to exposures to single PAHs. These unpredictable effects of the mixtures are not accounted for by environmental guidelines. Therefore, understanding the mechanistic features of PAH mixtures is a crucial research need.

The present work aimed to disclose the interaction effects of two PAHs, Phenanthrene and Benzo[a]pyrene that have different carcinogenic potentials and chemical structures. Then, to assess CYP1A1 protein levels, ZFL cells were exposed to Phe (3-ring PAH) and B[a]P (5-ring and carcinogenic PAH) individually and in mixture with 1:1, 1:2 and 2:1 ratios for 24h in Danio rerio hepatocyte cell line (ZFL cells). CYP1A1 levels were determined by Western Blot.

Results for CYP1A1 protein levels showed higher increase for BaP than for Phe. Furthermore, in the case of the mixture, an even greater increase was observed, which may suggest synergistic interactions.

The results are in line with previous investigations since it is known that BaP is an AhR agonist that regulates primary activation by CYP1A1. The increased results for the mixture suggest that Phe, even though it is a non-carcinogenic PAH, may further potentiate the toxic effects when mixed with a carcinogenic PAH. It is therefore clear, that the environmental guidelines need to be revised taking into account the compositions of the mixtures.

#### 4.06.P-Th290 Mixture Toxicity: Tannic Acid Impacts on Graphene Oxide and Pollutants Biological Effects

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When released into the environment, nanomaterials interact with the present pollutants altering their bioavailability and resulting in a complicated mixture of toxicity effects. Biomolecular coronas formed upon the nanomaterials release, significantly affect this process, competing with pollutants for surface interaction sites or multiplying the possibilities of interactions. This work comprehends an ongoing project that aims to develop a theoretical-experimental approach to understand the interactions between

graphene oxide (GO) and environmentally relevant molecules (i.e., organic matter and pollutants) and their impacts on ecotoxicity during co-exposure scenarios. Tannic acid (TA) is an environmentally abundant polyphenol with applications in developing novel technologies, such as nanomaterial synthesis, and their functionalizations. We found that TA has a significant dose-dependent mitigation effect on GO's toxicity over *Caenorhabditis elegans* at environment-relevant concentrations. To understand the mitigation effects of TA, we integrated experimental characterizations techniques (i.e., Atomic Force Microscopy, Raman, and X-ray photoelectron spectroscopy) to assess changes in the morphology and surface chemistry of GO. In addition, computational methodologies were employed to analyze the interaction mechanisms and GO's surface modification by TA. We performed a multilevel study with different theory levels; reactive classical molecular dynamics enabled the exploration of the chemical and conformational changes of TA and GO, whereas ab initio calculations provided information regarding the electronic properties of the system, such as the most reactive sites and their interactions. Further, we evaluated the TA and GO toxicity effects on the atrazine pesticide for the biological model Zebrafish. We found that GO and TA increase the toxic sublethal effects of atrazine, delaying embryo hatching, while TA also potentiated cardiac edema formation. However, when GO and TA are both combined with atrazine, the toxicity potentiation effect was not observed. Due to this multiplicity of responses raised from the complex environmental biomolecular interactions, integrating experimental and computational approaches is essential to understanding mechanistic aspects of mixtures toxicity, especially regarding nano-bio interactions. Here, we present how computational methodologies may be applied in the evaluation of nano-bio interface at molecular level.

#### **4.06.P-Th291 Sublethal Effect of an Environmentally Relevant Pollutant Mixture on Zebrafish Embryos**

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To measure the real extent of aquatic toxicity, there is a need to measure the actual toxicity effects of pollutant mixtures rather than single chemicals. Zebrafish (*Danio rerio*) play a key role in aquatic risk assessment throughout the globe, a procedure that usually involves exposing newly fertilized embryos to the desired pollutants at multiple concentrations. At the end of the exposure period, the toxicity is determined based on the outcome of the observations recorded, and the lethal dose (LC50/EC50) is calculated. Although effective, these experiments require excessive resources and are difficult to be used in a sustainable way. Therefore, cross-effective toxicity experiments, which accurately reflect aquatic toxicity while requiring manageable resources, are pursued. Furthermore, discovering sublethal effects of pollutant mixtures on the ecosystem is of high importance to protect and manage natural waters. The presented study aims to determine the actual toxicity of a pollutant mixture by establishing a feasible method for assessing sub-lethal toxicity of chemical mixtures on zebrafish embryos. Toxicity experiments are based on a pollutant mixture of 13 chemicals, derived from the Alexander micro-estuary, located in the eastern Mediterranean Sea. This representative mixture of pesticides and pharmaceuticals was established from a two-year study conducted in the Alexander estuary, a unique water body receiving municipal effluents and runoff from agricultural origins. This pollutant mixture, found to have a potential negative effect on the aquatic environment, is used in exposure experiments to zebrafish embryos and larvae. The experimental design aims to use small batches of embryos in a small range of concentrations with specific easy-to-find endpoints which reflect sublethal effects. The results are expected to reveal the real extent of the pollutant mixture toxicity to the Alexander estuarine ecosystem, while establishing an experimental design that will be easily implemented in additional aquatic environments.

#### **4.06.P-Th292 Air Particulate Mixtures from a Midsized Tropical City of Brazil Impacted by Biomass Burning: Concentrations of Polycyclic Aromatic Hydrocarbons and In Vitro Toxicity**

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In this study, atmospheric particulate matter (PM) samples were collected at Ribeirão Preto, an agro-industrial municipality with ca. 700,000 inhabitants located within the sugarcane belt of São Paulo State (Brazil). Although the use of fire for agricultural practices have been legislated in the State, a large number of accidental/criminal fires still break out every year, mostly during the dry season. The aim of this work was to investigate the impact of biomass burning on human health in Ribeirão Preto by determining the concentration of polycyclic aromatic hydrocarbons (PAH), their nitrated (NPAH) and oxygenated (OPAH) derivatives in the atmospheric PM, and evaluating the cytotoxic and genotoxic activity of the PM organic extracts using *in vitro* tests. Fractionated PM (cut sizes: 18, 10, 2.5 and 1  $\mu\text{m}$ ) was collected in a sub-urban and an urban site, from January 2020 to July 2021 ( $n = 19$ ). PAH and OPAH concentrations were higher in the dry season ( $2.59 \pm 1.72$  and  $0.68 \pm 0.69$   $\text{ng m}^{-3}$ , respectively) than in the wet season ( $0.28 \pm 0.20$  and  $0.07 \pm 0.06$   $\text{ng m}^{-3}$ , respectively), and correlated with fire *foci* number and with levoglucosan (a biomass burning marker), indicating that biomass burning is an important source of PAH and OPAH in the region. Conversely, no seasonal variability was observed for NPAH (dry:  $0.48 \pm 0.33$   $\text{ng m}^{-3}$  and wet:  $0.50 \pm 0.33$   $\text{ng m}^{-3}$ ), which did not correlate with PAH and levoglucosan, indicating that other sources, such as vehicular, could be more important to the emission of NPAH. The mean of benzo(a)pyrene equivalent index in the dry season was larger than the unit risk established by the World Health Organization, corresponding to an average excess lifetime lung cancer risk of 12 cases per 100,000 people. HepG2 cells were exposed to different organic extracts of the finest fraction ( $< 1 \mu\text{m}$ ) at the same concentration ( $1 \text{ m}^3 \text{ mL}^{-1}$ ). The dry season extract decreased cell viability (MTT assay) by 36% after 72 h of exposure, while no cytotoxic effect was observed for the wet season extract. Significant DNA damage (comet assay) was observed in cells exposed to the dry season extracts for 4 and 24 h, with an increase of up to 3-fold compared to the negative control. No genotoxic effect was observed for the wet season extract. Our results indicate that there is a health risk associated to air particulate mixture mainly associated with biomass burning in the region of Ribeirão Preto.

#### 4.06.P-Th293 Application of In Vitro New Approach Methodologies to Determine Whole Mixture-Based Relative Cancer Potency Factors

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Air pollution and airborne particulate matter (PM) are classified as carcinogenic to humans, but these complex mixtures of multiple compounds makes quantitative risk assessment a challenge. Current strategies for cancer risk assessment of air pollution are based on a pollutant-by-pollutant approach. This is clearly a simplification which excludes the possibility of interaction effects and may misestimate the actual cancer risk. Whole mixture-based testing using in vitro new approach methodologies (NAMs) has been suggested to facilitate the hazard and risk assessment of complex environmental mixtures. We have addressed this issue by developing a NAM for whole mixture-based cancer risk assessment of air pollution. The overall aim is to combine state-of-the-art methods for analysis of chemical composition of urban, diesel and biomass burning PM with in vitro testing of PM samples in order to determine Mixture Potency Factors (MPFs) estimating the carcinogenic potency of whole mixtures. Our results so far show that MPFs based on whole mixtures better indicate cancer potency than looking at single pollutants. Moreover, that these MPFs are in good agreement with potency values based on published data from *Salmonella* mutagenicity and in vivo carcinogenicity studies. This research will develop an approach that can be used for assessment of total carcinogenic effects of air PM pollution both for larger city-wide and for smaller site-specific risk assessments. Ultimately, this in vitro NAM will improve the cancer risk assessment of airborne PM by including the obtained knowledge about whole mixture potencies in already established models for estimation of lung cancer incidence in polluted environments.

#### 4.06.P-Th294 Source Apportionment and Health Impact of Atmospheric Particulate Matter in the City of São Carlos, Brazil

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Particulate matter (PM) is one of the principal causes of health problems worldwide.<sup>1</sup> These harmful health effects are related to the different compounds that are present in PM, some of which have known toxic, mutagenic and carcinogenic effects. To evaluate PM<sub>10</sub> sources and toxicity in lung cells, PM<sub>10</sub> samples were collected in São Carlos, a city located in the state of São Paulo (south-eastern Brazil), with ca. 240,000 inhabitants, 200,000 vehicles and an economy based on sugarcane production and diverse industrial activities. PM<sub>10</sub> concentrations ranged from 2.70–54.77 µg m<sup>-3</sup> (*n* = 47) values that were lower than the standard for 24h in Brazilian legislation, but the maximum value exceeded the limit recommended by the World Health Organization (WHO). Biomass burning plus secondary aerosols (40.7%) were the major sources of PM<sub>10</sub>, followed by the burning of biomass and fossil fuels (23.8%), biogenic emissions (19.7%) and soil (15.8%). PM<sub>10</sub>, Polycyclic aromatic hydrocarbons, and their nitrated and oxygenated derivatives, sugars, anhydrosugars and anions concentrations were higher in the dry season than in the wet season. This behaviour is explained by sugarcane burning and by reductions in precipitation levels, relative humidity, and air temperature. In 2016, 247,856 hectares of sugarcane plantations were burned to facilitate manual harvesting in the state.<sup>2</sup> Conversely, the sugar alcohol concentrations were higher in the wet season, and no seasonal variability was observed for cations. The mean benzo(a)pyrene equivalent index was greater than the unit risk established by WHO, corresponding to a mean excess lifetime lung cancer risk of 3.09 ± 4.23 cases per 100,000 people. Furthermore, exposure of A549 and MRC5 cells to PM<sub>10</sub> extracts for 24 and 72 h indicated that reductions in cell viability (MTT assay) were time-dependent and not dose-dependent. Reactive oxygen species assay (fluorogenic probe: 2',7'-dichlorodihydrofluorescein diacetate) results were consistent with the reductions in cell viability observed for A549 cells, indicating that cell death likely occurs following oxidative stress. Our results indicate that although PM<sub>10</sub> concentrations in São Carlos were not above the limits recommended by local legislation, there is a health risk associated with atmospheric particles.

#### 4.06.P-Th296 Identification of Key Toxicants in Municipal Wastewater Receiving Water Environments: Application of Effect-directed Analysis with In-situ Bioassay Samples

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Municipal wastewater is an important route for water pollution in urban environment. Conventional wastewater treatment processes often ignore the removal of contaminants of emerging concern (CECs), as a consequence, CECs may enter the receiving waters and threaten aquatic ecosystem security and even human health. Currently, water quality managements are mostly based on monitoring regular water parameters and limited number of priority pollutants, which may not be the main toxicity contributors in the mixtures. To identify bioavailable toxicants responsible for adverse effects from municipal wastewater receiving water and sediments, 10-d *in-situ* bioassays using tilapia and Asian clam were conducted in receiving waters of a municipal wastewater and adjacent urban waterways in Guangzhou, China. Two sites (the municipal wastewater receiving water (S1) and a sampling site in residential areas (S2)) that exhibited high toxicity to both organisms were selected for further effect-directed analysis (EDA) using the extracts of the biota samples and *in vitro* cell tests. Toxicity endpoints of the *in vitro* bioassays were selected based on adverse outcome pathways (AOP) and they showed the same toxicity trend as *in vivo* bioassays across sampling sites. Reversed-phase chromatography fractions of S1 and S2 were tested using *in vitro* bioassays, and the relatively hydrophobic fractions showed high responses on AOP26. Furthermore, target, suspect and non-target analysis of the toxicogenic



fractions were conducting by GC-MS/MS and LC-Q-ToF-MS. Some pharmaceuticals and personal care products (PPCPs), current-use pesticides (CUPs) and industrial compounds were identified in tissue samples and contributed significantly to the toxicity related to AOP26.

#### **4.06.P-Th297 A Vision for Safer Food Contact Materials and Articles: Public Health Concerns as Drivers for Improved Testing**

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Food contact materials (FCMs) are used to make food contact articles (FCAs) such as food packaging and food processing equipment, and they are ubiquitous in today's globalized food system. The chemical composition of FCAs is diverse and can be highly complex. Chemicals migrate from FCAs into foodstuffs, but current regulatory requirements are ineffective for protecting public health from hazardous food contact chemicals (FCCs) because they focus only on the individual starting substances used to make FCMs, and because they only assess genotoxicity as proxy for carcinogenicity. As a consequence, the non-intentionally added substances (NIAS) migrating from finished FCAs as complex mixtures are largely ignored. The current practice also implies that FCAs widely present on the market today are a source of hazardous chemicals and mixtures contributing to highly prevalent non-communicable diseases (NCDs) worldwide.

Based on the work with the Food Packaging Forum's Scientific Advisory Board, we propose here our vision of a novel approach for establishing the safety of FCCs in finished FCAs, thereby addressing (1) the overall migrate (i.e. mixture of all migrating FCCs), including unknown and/or untested NIAS, and (2) focusing testing efforts on those NCDs that are increasingly prevalent in the human population and that are associated with chemical exposures. We group these relevant NCDs into the Six Clusters Of Disease (SCOD) concept.

We envision an improvement of the current status quo of FCC testing by establishing cost-effective, rapid testing of all finished FCAs for their impacts on all relevant NCDs, as defined by the SCOD. Future research must focus on development of in-vitro assays that are sufficiently robust, relevant, and sensitive, and that are based on mechanistic information linked to the SCOD, for example from Adverse Outcome Pathways and the Key Characteristics of Toxicants concept. Taken together, this vision for a novel approach offers a significant improvement to the status quo because it offers better protection of public health by contributing to the prevention of NCDs that are associated with all chemical exposures from FCAs, including mixtures containing unknown NIAS.

#### **4.06.P-Th298 Predicting the Chemical Properties and Exposure of Mixtures Using QSPRs and PPLFERs**

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Developing a better understanding and improved predictive tools for mixtures has been identified as a key research need in the field of chemical risk assessment. Poly-parameter Linear Free Energy Relationships (PPLFERs) are a well-established tool in environmental chemistry for predicting partitioning and transport properties of chemicals. PPLFERs are composed of system parameters which characterize the solvation environment in which partitioning takes place, and solute descriptors which characterize the chemical of interest. Quantitative-Structure Property Relationships (QSPRs) have previously been developed for PPLFER solute descriptors and system parameters which allow them to be applied to a wide range of liquid mixture phases. A new model for predicting the Vapor Liquid Equilibrium (VLE) of mixtures has been developed based on a modification of the log-linear co-solvency model of Yalkowsky with the solute descriptors and system parameters of the mixture components as inputs. This new VLE model is then used as an input for a new model for predicting the skin permeability ( $K_p$ ) of mixtures applied to human skin. The  $K_p$  model also accounts for penetration enhancement effects that can be attributed to partitioning. The models are applied to assess two different mixture exposure scenarios, a petrochemical spill and application of skin cream to the skin. These tools have been made available as a part of the freely available on-line Exposure And Safety Estimation (EAS-E) Suite platform ([www.eas-e-suite.com](http://www.eas-e-suite.com)).

#### **4.06.P-Th299 Chronic Metal-organic Mixture Toxicity: Quantitative Reappraisal and Identifications of Data Gaps**

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In the Chemicals Strategy for Sustainability, the EU Commission calls to systematically integrate the issue of combined exposure into chemical risk assessments. Ecological risks of chemical mixtures have predominantly been studied within broad substance groups, (e.g., metals, pesticides), while chronic effects of metal-organic mixtures have rarely been assessed. The metal-organic

mixture project within the Metals Environmental Exposure Data Program (MEED) aims to provide scientific evidence on effects of metal-organic mixtures. As a first step, a literature review and quantitative reappraisal of chronic mixture toxicity of metals and organic chemicals was conducted. The null hypothesis was that metals and organic chemicals act independently from each other, i.e., that the Independent Action (IA) model is an accurate predictor of mixture toxicity, and that it is on average more accurate than the concentration addition (CA) model.

Overall, focusing on the period 2007-early 2022, we identified only 4 chronic metal-organic mixture toxicity studies (13 individual experiments) that were of sufficient quality and relevance to be included into the systematic quantitative reappraisal. Those studies were dominated by tests with algae and with Cu, with limited other species and metals tested. Among these few studies, IA performed somewhat better than CA in predicting metal-organic mixture toxicity (lowest root mean square error), whereas CA was generally the most conservative (i.e. overestimates mixture effects more than IA). At low effect levels (10% mixture effect), CA performed relatively well, albeit some experiments suggested quite strong synergisms. However, these synergisms were unreliable and/or observed at unrealistic exposure concentrations (based on monitoring data reported in the Waterbase environmental monitoring database). In fact, most identified chronic metal-organic mixture studies were conducted outside of environmentally and/or regulatory relevant mixture concentrations, which means that it is not appropriate to draw any meaningful or general conclusions with respect to our central question whether IA is a better model to predict metal-organic mixture toxicity than CA, and how protective CA would be on average. Hence, there is a need to investigate metal-organic mixture toxicity at environmentally and regulatory relevant concentrations, with appropriately sensitive species and endpoints.

#### **4.06.P-Th300 A Proposed Higher Tier Refinement for Lagomorphs Exposed to a Dual Active Plant Protection Product Applied to Cereals**

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Higher tier refinements are often required in order to pass mammal risk assessments for single-active pesticide products, and the complexity of these refinements often increase when products contain more than one active substance. This work describes risk assessment of a pesticide product containing two active substances, which is applied in the early growth stages of cereals. The screening and Tier 1 risk assessments of the individual active substances performed according to EFSA/1438/2009 indicated a long term risk to the indicator species "large herbivorous mammal (lagomorph)" from one of the active substances in the product, and also from the combined long-term risk assessment. Refinement options that addressed not only the individual substance risk, but also the combined risk were therefore required. Refinement of the focal species and diet were performed via publicly available literature sources. Refined exposure calculations were then performed for each active substance before performing a refined combined assessment. The result was that both the individual and the combined assessments met the long term TER criteria. The presentation of this information is relevant as it demonstrates the relevance of using a variety of literature data to refine pesticide product risk assessments as a potential alternative to field studies.

#### **4.06.P-Th301 The Aquatic MixTox Tool for Risk Assessment of Plant Protection Products and the Related FAQ Document**

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In the EU aquatic risk assessment the authorisation of a plant protection product with more than one active substance requires a risk calculation considering the toxicity of the mixture. A scheme and background information are provided in the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290; AGD).

The authors developed the i.e., an Excel-based calculation tool composed of different worksheets that provides a calculator and user guidance following the scheme of the AGD. It is intended to support a relatively complex process by harmonising the assessment and facilitating the delivery of appropriate and consistent risk calculations. The tool has been available to all European pesticide risk assessment stakeholders including EU Member States since early 2021 and was presented at SETAC 2022 (Hillebrand et al., 2022). An updated version has been released in December 2022.

In addition to the tool itself, a new FAQ document is also available that complements the excel spreadsheets. This living FAQ document includes recommendations on several issues that emerged during developing the tool. It informs on the lessons-learned and goes where necessary beyond the advice provided in the AGD. The various issues detailed in the FAQ document include currently clarifications on; 1. the (mixture) assessment of metabolites, 2. the combination of different FOCUS Steps for different assessment steps of the AGD scheme, 3. the inclusion of chronic mixture toxicity and 4. the driver assessment.

#### **4.06.P-Th302 Evidence from In-situ Bioassays and Suspect Analysis Distinguished Region-specific Aquatic Toxicity Characteristics Across China**

*Yujun Tong, Huizhen Li, Fei Cheng, Yuanyuan Pei and Jing You, Jinan University, China*

Aquatic environment presents a combined pollution status with complex mixtures, which hindered accurate assessment of ecological risk using methods based only on chemical analysis or laboratory toxicity tests. Alternatively, *in-situ* bioassays measure the stresses suffered by aquatic organisms in real environments and may provide more environmentally-relevant results. Two native species (pelagic Chinese rare minnow and benthic Asian clam) were applied for 10-d *in-situ* bioassays in three basins with economic gradient in China, including the Pearl River, Taihu Lake and Poyang Lake basins. Eventually, aquatic risk in these regions was prioritized using a weight-of-evidence method, with chemical residues (98 target and 942 non-target

contaminants) and in situ biological effects (survival and enzymatic activities) as lines of evidence. Survival of the tested organisms was significantly reduced at all sites, while significant spatial variability was noted for various enzymes. A diversity of chemicals was detected in aquatic environments, showing region-specific patterns. The combination of exposure and effect data showed that both water and sediments in the highly developed Pearl River basin had the highest ecological risk, while high risk was also shown in the sediments of the agriculture-dominated Poyang Lake basin. The results of risk assessment in different economic zones show the generality of the *in-situ* bioassay method, and emphasize that biological effects should be considered when conducting risk assessment.

#### 4.06.P-Th303 The Long-term Ecological Effects of Single Antibiotics and Their Mixtures on Freshwater Ecosystems

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The wide and large use of antibiotics all over the world increased the residue concentrations of antibiotics in environments, where the organisms are exposed continuously to multiple antibiotics. However, the knowledge about the effects of chronic exposure of antibiotics on aquatic ecosystems and the effects of their mixtures is sparse. Hence, in this study, we conducted an indoor microcosm experiment to mimic real aquatic ecosystems to investigate the effects of long-term exposure to antibiotics on aquatic ecosystems. Sulfamethazine (SMT) and tetracycline (TC) were selected as representative antibiotics which were applied to tested microcosms with different concentrations (SMT: 0.1 ug/L, 1.0 ug/L and 10 ug/L; TC: 1.5 ug/L, 15 ug/L and 150 ug/L) and combinations. After 28 days (4 weeks) of exposure, the results showed that the concentration of green algae was lower in the two higher concentration treatments of both STM and TC compared to the control until the end of the experiment while no significant difference between the different treatments was observed in the mixture treatments. For the zooplankton community, their abundance and diversity were significantly affected by the antibiotics and their mixture. The total abundance of zooplankton in all highest concentrations of three different treatments (SMT, TC and mixture) increased substantially in the first 7 days or first 14 days, followed by a decrease until the end of the experiment. Moreover, the microbial degradation didn't present significant differences in the first 14 days but showed significant effects of the treatments after 28 days of exposure. The abundance of macroinvertebrates (*Asellus aquaticus*, *Gammarus pulex*, *Physella sp.* and *Planorbis sp.*) didn't show a clear significant treatment related difference during the experiment period but the food consumption by them indicated that in the SMT treatments food consumption was higher in the highest concentration (10 ug/L) than that in the lowest concentration (0.1 ug/L) ( $p < 0.05$ ). The results from this study showed that the selected antibiotics pose long-term effects on aquatic ecosystems, and The mixture of the antibiotics resulted in different effects than the individual antibiotics, which will be studied in more detail the coming months.

#### 4.06.P-Th304 Which Organic Substances Occur Most Frequently in Hazardous and Environmentally Relevant Binary Mixtures with Metals in European Freshwater?

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Ecological risks of chemical mixtures are predominantly assessed within a single compound group rather than simultaneously accounting for different groups of chemicals. Few studies are available describing the chronic mixture toxicity of metals and organic pollutants and most of these studies have been conducted at concentrations that have neither environmental nor regulatory relevance. This study aims to prioritize organic substances that potentially pose a aquatic mixture risk in binary pairs with metals. The analysis was based on monitoring data from the European Environmental Agency Waterbase. 16 metals considered in this prioritization had all been identified previously as inorganic substances potentially contributing to aquatic mixture risks. All organic substances with an individual CAS number, present in the Waterbase, were included in the analysis (598). 418 PNECs were derived from NORMAN's PNEC database, 29 from the Swiss Ecotox Center water quality parameters database, 84 PNECs were calculated using the Envirotoxdatabase.org database. 68 compounds were excluded because no PNEC could be found in any of the consulted databases, leaving 531 compounds for the analysis. 512 organics were measured together with at least one of the 16 metals. To priority-rank the organic substances, we calculated the percentage of mixtures at risk – based on the Hazard quotient ( $HQ = PEC/PNEC$ ) – for each metal-organic pair. This percentage was calculated by dividing the number of samples showing risk (sum  $HQ > 1$ ) where both the organic and metal contribute at least 10% to the risk by the number of samples where both compounds were measured together. The number of metals (out of 16) for which % mixture risk was  $>10\%$  (priority) were counted and ranked in decreasing order. The (preliminary) top priority organic compounds belonged to various substance groups with different modes of action such as pesticides (imidacloprid), pharmaceuticals (diclofenac), Industrial chemicals (PAHs, Bisphenol-A). This prioritization identified realistic metal-organic mixtures driving the risk to aquatic communities in the field based on PNECs. It is possible, though, that this prioritisation may be somewhat biased to include more organic substances for which high assessment factors have been used in deriving their PNECs. Further laboratory test will be conducted to test the occurrence of mixture interactions between metals and organic substances in some of these identified priority mixtures.

#### 4.06.P-Th305 Analysis of the Contribution of Surfactants to Mixture Toxicity in French Rivers

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Surfactants are used in many industrial applications as well as in “down-the-drain” household cleaning and personal care products. Their high production volumes and use can lead to concentrations in local water bodies that potentially exceed threshold values. A recently published monitoring study reported detection of several surfactants in French surface and waste waters, with

some exceeding predicted no effect concentrations, indicating that these substances might require further attention. In the environment, chemicals mostly occur in complex mixtures. The objective of the current study was therefore to assess the potential risk of surfactants in French river basins based on their relative contribution to complex mixture effects, rather than on single-compound effects. Surface water monitoring data were gathered from two databases and data from 234 water samples with 995 targeted chemicals (including 13 surfactants) from 80 locations in France were used in the mixture analysis. For each chemical, the effect concentration (hazardous concentrations for 5% of species, HC5) was collated from a specific database or if needed, calculated from ecotox data in the EU REACH registration dossier. To characterize mixture toxicity, hazard indices (HI) were calculated for each mixture by summing hazard quotients ( $HQ = \text{measured environmental concentration}/HC5$ ) of each mixture constituent. The highest number of surfactants quantified in the same sample was six and linear alkylbenzene sulfonates were the most frequently quantified surfactants in the monitoring dataset (84% of samples). Most surfactants were found at low concentrations, with only laurylpyridinium demonstrating exceedance of the HC5 value. Specific evaluation of this data, however, demonstrated that the HC5 generated for this substance was below the quantification limit, artificially increasing the HI. According to the concentration-addition model assumption, the mixture toxicity was unaffected when surfactants were excluded from the mixture. Overall, this study demonstrates that surfactants are unlikely to significantly contribute to the mixture toxicity in surface water systems. Furthermore, it highlights that the choice and quality of the threshold value is critical for the conclusion of the risk assessment.

#### 4.06.P-Th306 Precision Environmental Health: A Framework For Identifying Chemical Components of Concern in Natural Rivers

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In a natural river, hundreds to thousands of chemicals co-exist as complex mixtures, which pose potential health threats to aquatic organisms and humans. Identifying and evaluating the effect of each chemical component in the environment is undoubtedly an insurmountable challenge to ecotoxicological studies and an unrealistic approach to identifying the harmful component driving the mixture effect at environmentally relevant concentrations. Thanks to the advancements in “omics” technologies and machine learning algorithms, subtle variations in biomolecular levels can be captured for extracting the biomolecular signatures of chemical exposure from the systematic profiles in a toxicogenomic study.

Here, we propose the **Precision Environmental Health** framework to establish the associations between the chemical components within the environmental mixture and their co-responsive biomolecular signatures. Our framework re-invents the use of sentinel organisms to identify **putative molecular key events (mKEs)** activated by the chemical components within the environmental mixtures and associated with potential hazards. The framework relies on gene orthologs within conserved pathways of toxicity among animals to enable cross-species extrapolation for the early diagnosis of the potential hazards of chemical pollution, even when chemicals are present at environmental, usually sublethal, concentrations.

We conducted a case study with local-scale sampling along the Chaobai River as a proof of concept. By exposing the ecotoxicological model species (*Daphnia*) to the ambient river samples, the changes in gene expressions reveal the overall biochemical and physiological/metabolic responses to chemical pollutants. With network analysis and multi-block correlation analysis, chemical components with potential hazards are identified and suggested by the functionally conserved pathways enriched in the associated putative mKEs. This case study demonstrates that the chemical components of concern are identified based on establishing links between bioactive chemicals within mixtures and the perturbations of functional pathways under the Precision Environmental Health framework.

#### 4.06.P-Th307 Sensitivity of Different Phytoplankton Species to Chemical Activity of Mixtures

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Chemical activity is a thermodynamic concept based on the chemical's energetic state. Under equilibrium, the measured activity is the same in all phases regardless of the quality of the phase. Therefore, the natural variability in lipid composition among species might modulate the total amount of HOCs in the cell, but not impact the chemical activity. Still, some phytoplankton species are more sensitive than others to HOC mixtures. This project aims to further investigate the sensitivity of different phytoplankton species to chemical activity. Saturated solutions of acenaphthene, fluorene, phenanthrene and fluoranthene were prepared in methanol and combined to produce mixtures in a range of chemical activities (0.008 – 0.13). Chemical activity and passive dosing were integrated into an algae toxicity test, where silicone was loaded with the PAH mixture serving as a donor to the system. The effect of growth inhibition on six phytoplankton species (*Rhodomonas salina*, *Phaeodactylum tricornutum*, *Heterocapsa triquetra*, *Monoraphidium minutum*, *Prymnesium parvum* and Picocaryote species). Both medium and biota samples were analyzed through LC and GC-MS methods for exposure confirmation. Biota samples were collected for total lipids and lipid profiling. The results from *Rhodomonas salina* show that the concentrations of PAHs in the algae, except fluorene, increased proportionally with increased chemical activity. Individual PAHs contributed similarly to the exposure ( $25 \pm 5\%$  to the total chemical activity), which indicates the efficiency of the passive dosing method. Chemical activity and the growth inhibition followed a dose-response curve. As a result, the effect on single cells is reflected in the population growth. The effective activity

that causes 50% effect was 0.067. Based on this endpoint, *R. salina* would be considered more tolerant than *Daphnia magna* ( $Ea_{50} = 0.036$ ), and more sensitive than the marine diatom *P. tricornutum* ( $Ea_{50} = 0.14$ ), to chemical activity. The remaining phytoplankton species have been exposed to the same mixture and the next step is to determine the activities in both medium and biota. This study provides confirmatory evidence that the chemical activity concept can be used to assess the mixture effects of Hydrophobic Organic Chemicals (HOCs) on the toxicity test. This is an ongoing experiment, and the sensitivity of the other phytoplankton species and lipid characterization are being evaluated for further comparisons.

#### **4.06.P-Th308 The Effects of Long-term Exposure of Bio-ozone Treated Wastewater Treatment Plant Effluent on the Structure and Functioning of Freshwater Microcosms**

**Paul van den Brink**, Myrthe van Venetië, Lara Schuijt, Koen van Gijn, Alette Langenhoff and Dailing Wu, Wageningen University & Research, Netherlands

Freshwater ecosystems are constantly subjected to various anthropogenic pressures, such as pollution of pharmaceuticals and personal care products. These substances enter the systems via effluent of wastewater treatment plants, as these are not yet designed to remove the substances. The aim of this study was therefore to investigate whether long-term exposure (6 weeks) of 8 different exposure solutions (wastewater treatment plant effluent treated with bio-ozone processes) affects the structure and function of the aquatic freshwater ecosystem in an outdoor microcosm experiment, focussing on several invertebrate populations. The experimental set-up contained 100 microcosms filled with approximately 58 L of water (29 L supply basin water and 29 L of 1 of the 8 exposure solutions), sediment and organisms. In total 9 different treatments were added to the microcosms (one control group and 8 different exposure solutions (wastewater, spiked wastewater, wastewater treated with bioreactor processes and wastewater treated with ozonation processes)). The micropollutant concentrations in the wastewater is probably too low to have a significant negative effect on the structure and functioning of freshwater ecosystems, although the increase in algae could be a direct effect of nutrient addition through the wastewater or an indirect effect due to a reduction in grazing pressure. Furthermore, the ozonation processes is more effective in removing micropollutants from the wastewater in comparison with the bioreactor processes. Here, a higher ozonation intensity also seems to be more effective. Lastly, the results give an indication that the ozonation processes in some cases produce other toxic by-products that negatively affects the structure and functioning of the freshwater aquatic ecosystem. It is recommended to conduct further research into the possible formation of toxic by-products due to ozonation processes and their effect on the aquatic ecosystem.

#### **4.06.P-Th309 Wastewater Monitoring Based on an Epidemiological Method for Assessing Environmental Mixture Risks of Anti-Tuberculosis Drugs**

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The environmentally relevant concentration of antimicrobial agents can be monitored by surveying or predicting the infected population in communities. Our study proposed real-time environmental monitoring based on epidemiology for assessing environmental mixture risks of anti-tuberculosis (TB) drugs, isoniazid (INH), rifampicin (RMP), and ethambutol (EMB). The trends of environmentally relevant concentration are decreasing in 2020 – 2030. The EMB has the highest residues in the environmental releases, whereas the INH was the lowest. For the mixture toxicity threshold, the no-observed effect concentration (NOEC) of RMP+EMB and INH+RMP+EMB mixture exposure are not observed from daphnia (*Daphnia magna*) acute immobilization test, whereas the LOECs is 70 mg L<sup>-1</sup> and 76.26 mg L<sup>-1</sup>, respectively. The NOEC was 26.25 mg L<sup>-1</sup> and 112.5 mg L<sup>-1</sup> to INH+RMP and INH+EMB, respectively, higher than the NOEC of individual anti-TB drugs, indicating the antagonistic effects of INH+RMP and INH+EMB. The approach of combination index was used to examine the mixture toxicity in response to levels of immobilization. RMP+EMP range from 1.27 – 1.33, showing moderate antagonism. Other binary mixtures are higher than the RMP+EMP that presented antagonism, and the ternary mixture tends to be a nearly additive effect when exposing high concentrations of anti-TB drugs. The predicted environmental concentration was used to calculate the environmental exposure risk of anti-TB drugs in individuals and mixtures would decrease from 2020 to 2030; risk quotients were lower than 1, indicating no environmental risk concerns.

#### **4.06.P-Th310 Effect Based Monitoring of the Effluent of a Public Wastewater Treatment Using Cyanobacteria *Microcystis Aeruginosa***

**Warich Leekitratapanisan** and Karel De Schamphelaere, Ghent University - GhEnToxLab, Belgium

Public wastewater treatment plant (WWTP) effluents are a major source of organic micropollutants (OMPs) released into surface water. This could pose an impact on the aquatic ecosystem. Screening-level environmental risk assessment is a tool to identify problematic compounds as well as hot spots of contamination. However, the drawback could arise from the lack of measured effect data and analytical methods of detection for these chemical cocktails as well as transformation products. To minimize these issues, the effect-based method is a holistic approach, which captures the toxicity of the whole chemical mixture. In Flanders, Belgium, more than 30 public WWTP effluents are at risk according to the calculated sum of PNEC-based risk quotients and toxic units of three biological quality elements (sumRQ-PNEC, and sumTUs of algae, crustacean, and fish >1) based on 5-year OMPs monitoring data by Flemish Environment Agency. In this study, we selected 18 high-predicted environmental risks to conduct effect-based monitoring using cyanobacteria *Microcystis aeruginosa* for validation of ERA. The effluents were concentrated using OASIS HLB cartridges and their toxicity was tested based on OECD201. The growth inhibition of cyanobacteria was pre-screened at a relative enrichment factor (REF) of 10. All WWTP extracts inhibited the cyanobacteria growth by more than 10% and four of them had growth inhibition of more than 50% at a REF=10. The results of the pre-screening test exhibited a moderate to high correlation with the sum of RQ-PNEC and TU algae (Pearson's  $r$  of 0.59 and 0.74,

respectively). The results support the usefulness of environmental risk assessment to identify the problematic sites posing environmental risks. For future work, we are planning to identify the main contributors to the toxicity of cyanobacteria using effect-directed analysis (EDA). The EDA approach will be done by utilizing the fractionation process, and toxicity testing in complement with chemical analysis to narrow down the problematic chemical mixtures.

#### 4.06.V Complex Mixtures in Chemical Risk Assessment: Challenges and Opportunities

##### 4.06.V-01 Effect Based Monitoring in Ecosystems Potentially Affected by Power Plants

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Power plants for regasification of liquefied natural gas (LNG) have been proposed with the aim to satisfy the energy demand. However, it is necessary to control the potential emissions, releases and losses of chemical pollutants in the ecosystems where these plants are located. In the context of the Health Impact Assessment an ecotoxicological monitoring has been recommended for preventing the possible adverse effects on the ecosystems and human health. Effect Based Methods (EBM) will be applied to this aim in marine waters and sediments, but also in surface waters and soils located in the proximity of the pipelines for the gas furnitures. EBM are key tools for the detection of the effects caused by chemical mixtures and contaminants which are not included in the legislation. The EBM that are suggested are both *in vivo* and *in vitro* bioassays, and will cover several endpoints, including genotoxicity and embryotoxicity. Furthermore, such methods will be performed in the phases of scoping and monitoring of the health impact assessment. EBM will play an important function in the early warning system and, at the same time, they will play a screening role for further chemical analyses. We consider that applying monitoring based on EBM to gather information on the status of the ecosystems potentially affected by the impacts of LNG regasification power plants, can help to protect the ecosystems and the health of the population living in these areas.

#### 4.07 Environmental Risk Assessment of Organic and Inorganic UV filters

##### 4.07.P-Mo322 Assessing Environmental Hazard of Sunscreen Formulations for the Development of Safe(r) and Sustainable by Design Products

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Every year, tons of sunscreen end up in the ocean raising emerging concerns regarding the risks to the environment. In sunscreen formulations, UV filters, either organic or inorganic, are major active ingredients present at high concentrations. Some of these UV filters are known to cause harmful effects on marine organisms. For this reason, several authorities have banned specific UV filters and are now supporting the development of more eco-friendly, safer and approved alternatives. In this context, the objective of this work was to assess the environmental hazard of different organic and inorganic UV filters to support the development of Safe(r) and Sustainable by Design (SSbD) sunscreen formulations. In this work, we tested the organic filter ethylhexyl methoxycinnamate (EHMC) and the mineral filters ZnO, nano-ZnO, TiO<sub>2</sub>, US (ZnO, TiO<sub>2</sub>, SiO<sub>2</sub>), UT (TiO<sub>2</sub>, SiO<sub>2</sub>) and UTM (FeOOH, Fe<sub>2</sub>O<sub>3</sub>, Fe<sub>3</sub>O<sub>4</sub>, TiO<sub>2</sub>, SiO<sub>2</sub>). For it, we carried out the acute ecotoxicological tests: luminescent bacteria test, microalgae growth inhibition test and the *Artemia sp.* nauplii immobilisation test. In addition to that, inorganic filters were characterised using SEM-EDX, DLS and ICP-MS for particle's dissolution in marine water (MW). Based on the SEM-EDX analysis, ZnO and nano-ZnO samples showed irregular particles of 211.1 to 287nm and 79.10 to 87.89nm respectively. TiO<sub>2</sub> samples showed spherical particles of 175.8 to 181nm in size. US, UT and UTM were composed of irregular particles of 158.2 to 738.5nm. DLS analysis showed that all samples aggregate and showed negative surface charge in MW. ICP-MS analysis indicated that predominantly samples containing Zn were less stable in MW. Luminescent bacteria test showed that EHMC was the most toxic UV filter tested, followed by nano-ZnO, ZnO and the UV filter mixture US. The microalgae growth inhibition test indicated that all UV filters tested inhibited cell growth. The *Artemia sp.* nauplii immobilisation test showed that EHMC was the most toxic UV filter tested, followed by nano-ZnO, ZnO and the UV filter mixture US. As conclusions, results indicated that ZnO and nano-ZnO are important contributors to the toxicity of inorganic UV filters, being nano-ZnO more toxic than ZnO. Taken together, results indicated that toxicity of UV filters was as follows: EHMC>nano-ZnO>ZnO>TiO<sub>2</sub>>US>UTM>UT. Thus, in the SSbD context, safe(r) sunscreen formulations should avoid EHMC and Zn in their formulation.

##### 4.07.P-Mo323 Primary and Ultimate Biodegradation of Benzophenone-type UV Filters Under Different Environmental Conditions and the Underlying Structure-biodegradability Relationships

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Although benzophenone-based (BP) UV filters are widely used in different branches of industry information about their biodegradation under environmentally relevant conditions is scarce. We investigated ten common BP-based UV filters, sharing the same basic structure and differing only in their substituents, with respect to their primary and ultimate biodegradability. The primary biodegradation of the selected BPs was studied in river water at environmentally relevant concentrations (1 µg/L) while varying specific, crucial environmental conditions. Moreover, their ultimate biodegradation was examined according to OECD guideline 301 F. The range of applied complementary test procedures allowed to correlate the biodegradability of BPs to their structural features. Thereby, test conditions (aerobic, suboxic, supplementation of nutrients) seem to have a minor influence on

the biodegradation potential of the studied BPs. Commonly used ultimate biodegradability tests, according to OECD guideline 301, offer a rapid option for the identification of readily biodegradable derivatives, such as BP-1 and BP-3, or inherently biodegradable compounds, such as BP, BP-4 and BP 7. Complementing these approaches with methods that also provide structural details, such as LC-MS/MS, may provide additional knowledge about the primary (though not ultimately biodegradable) derivatives. Concerning the structural dependence of the biodegradability of BPs, features such as hydrophobicity or the prevalence of certain functional groups were not the determining factors. Instead, the substitution pattern plays a crucial role. This hypothesis was supported by docking simulations showing systematic differences in the orientation of BPs within the active site of a cytochrome P450 enzyme. The basic structure BP and derivatives that carry substituents on only one of the two rings, such as BP-1, -3, -4, and -7, can be readily biodegraded (> 50%) within 28 d. One exception is BP-10, which is very heterogeneously substituted on both rings and thus different orientations within the active site of CYP 450 are possible. This oral presentation provides an insight into the aquatic persistency of various BPs, which is highly relevant for the environmental hazard assessment of this class of substances.

#### **4.07.P-Mo324 UV Filters in the Environment: Using Market Research Methods to Fill Exposure Data Gaps**

**Andrea Carrao<sup>1</sup>**, James C Coleman II<sup>1</sup> and Harshita Kumari<sup>2</sup>, (1)Kao USA, (2)University of Cincinnati, James L. Winkle College of Pharmacy

A growing number of scientific and media articles have investigated the impact of sunscreen active ingredients (ultraviolet (UV) filters) on environmental health. While the effects of UV filters in the aquatic environment are well researched, environmental exposures are often understudied. Consumer sunscreen usage habits directly influence the amount of sunscreen products, and subsequently UV filters, released into the environment. These habits also directly impact environmental exposure assessments of UV filters.

Traditional market research tools, such as online surveys and clinical studies, can be repurposed for scientific research thus informing challenging environmental safety questions. These methods can be used to address data gaps in environmental exposure assessments, particularly when normal product use results in direct release to the environment, as is the case with typical sunscreen products. Results from a recent large-scale online consumer survey will be presented as well as several key learnings. In addition, a home usage study designed to measure consumer sunscreen usage over a four-week period will be presented along with results from a sunscreen rinse-off study conducted in human volunteers. Taken together, these data will help refine exposure assessments of UV filters, making it possible to predict more realistic environmental concentrations. This research is critical to understanding the real environmental impact of UV filters.

Sunscreen products protect consumers from the harmful effects of solar radiation. Therefore, it is important to ensure environmental risk management decisions regarding UV filters in sunscreens are well-informed and do not unnecessarily result in reduced availability of a significant form of sun protection.

#### **4.07.T-01 Review of Fate, Exposure, and Effects of U.S. Sunscreens in Aquatic Environments and Implications for Sunscreen Usage and Human Health**

**Charlie Menzie**, *Ecological Sciences, Exponent*

Concerns have been raised about the potential toxicity of sunscreens to a variety of marine and freshwater aquatic organisms, particularly corals. At the same time, there are concerns that people will use less sunscreen as a result of environmental concerns. An ad hoc committee of the National Academies of Sciences, Engineering, and Medicine reviewed the state of the science on the use of the active ingredients in sunscreens (UV filters) currently marketed in the United States. This review was conducted to provide information useful for future application in ecological risk assessments with an assessment of available information on UV filter fates, exposure, and effects. Based on their review, the committee recommended that ecological risk assessments for sunscreen active ingredients used in the United States be conducted by U.S. Environmental Protection Agency. The report also includes review of the potential human impacts that could result from changes in availability of certain UV filters for use in sunscreens, in order to inform management of both human and ecosystem health. This presentation will describe the committee's findings, conclusions, and recommendations related to this issue, as well as the priority knowledge gaps to fill to inform higher tiered risk assessments. The presentation will include discussion of the intersection of aquatic chemistry, environmental toxicology, ecology, and epidemiology to understanding the potential for risks from UV filters and implications to human health for changes in sunscreen use.

#### **4.07.T-02 Spatial and Temporal Investigation of Concentrations of Organic UV Filters in Seawater from The Florida Keys, USA**

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Despite concerns regarding the impact of UV filters contained in sunscreens to corals there is limited or no information available on the environmental concentrations of these chemicals in seawater from coral reefs in Florida. Previous studies from other locations have found spatial and temporal variations in concentrations, for example, lower concentrations with depth in the water column, distance from the shoreline and in times of lower recreational activity. Researchers have hypothesized that concentrations are higher in the microlayer yet there is limited or no data to support this. To address these knowledge gaps seawater samples from multiple sites (i.e. beach and reef locations) in the Florida Keys, USA were analyzed for 13 organic UV filters. At recreational beach locations multiple sites (and replicate samples) were assessed for concentrations in the microlayer and surface seawater at two distances from the shoreline. A temporal assessment was conducted with samples collected early in the morning,

mid-afternoon and late in the evening. At reef locations sites (and samples) seawater both surface and seawater collected at coral depth were analyzed. UV-filters were measured using LC-ESI-MS/MS techniques in both the dissolved and particulate fractions. Numerous UV filters were detected in seawater with the highest concentrations present in the sites closest to the beach correlating with the level of recreational activity. UV filters were present at significantly lower concentrations in samples taken further from the beach and in early morning and late evening samples. A number of UV filters were present in higher concentrations in the microlayer samples compared with the corresponding surface water samples. A number of UV filters were detected in seawater collected both at coral depth and the surface, although both were at much lower concentrations compared to beach samples. This study provides insight as to the environmentally relevant concentrations in seawater samples at beaches and coral reefs in the Florida Keys. Furthermore, the spatial and temporal assessments provide data that are essential for developing probabilistic marine exposure models and environmental risk assessments.

#### **4.07.T-03 Model Simulations of UV Filter Exposure to Marine and Freshwater Organisms**

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Sunscreens are vital in preventing skin cancer by absorbing harmful UV-A and UV-B light. Questions have been raised regarding the potential impact of specific UV filter ingredients in aquatic systems. Given the lack of robust and high-quality monitoring data to characterize UV filter concentrations in water and sediment under the diverse conditions in which sunscreens are used, a modeling framework is being developed to evaluate the behavior and exposure concentrations of different UV filter ingredients under high exposure beach scenarios. The modeling framework consists of several levels ranging from simplistic dilution-based estimates to more complex 3-dimensional circulation models. Hydrodynamics play an important part in UV filter dispersion; therefore, scenarios and sites identified for benchmarking model performance represent a variety of aquatic environments, including highly-flushed, semi-flushed, and more static waterbodies. Rarely do monitoring studies record information on all input parameters required for model setup; thus, certain inputs are based by necessity on assumptions. In particular, site-specific information necessary to estimate UV ingredient emissions is often unavailable, including the specific sunscreen products used, consumer application and reapplication patterns, and swimming behavior with respect to time in water and extent of immersion in water. To account for the uncertainties around these inputs, the UV ingredient emission estimate is addressed in a probabilistic manner. The modelling framework was applied to different sites for benchmarking and ultimately scenarios were created to represent various environmental conditions where UV-filters are used to evaluate potential ecological risk. This presentation describes the framework, comparisons of model predictions to monitoring data for three study sites, and the development of model scenarios to evaluate the potential exposure of UV filter ingredients under a diverse range of highly populated beach environments.

#### **4.07.T-04 Developing Relevant and Reliable Toxicity Assays in the Scleractinian Coral *Acropora cervicornis***

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Coral reefs are unique ecosystems supporting a significant diversity of marine life in tropical regions. Coral reefs and associated organisms are directly or indirectly negatively impacted by a combination of stressors including a diverse array of chemical contaminants. Additionally the impacts of chemical contaminants can be enhanced when combined with co-stressors, including UV radiation and those relating to climate change, such as ocean warming and acidification. Recently, concerns have been raised regarding the impact of UV filter constituents contained in sunscreens and other products. However, clear assessment of the environmental risk of chemical contaminants to scleractinian corals is hampered as there are currently no standard test protocols for corals (i.e., OECD, US EPA), and it is unclear how representative other standard test species (i.e. marine invertebrates and algal species) may be, given the complexity of the host-symbiont-holobiont system. Toxicity tests with corals have been conducted but the lack of a standard methodology has limited comparisons between studies and highlighted concerns on data reliability and quality. Building upon experiences conducting coral and other cnidarian toxicity tests with numerous chemical contaminants of diverse physical-chemical properties we provide guidelines and discuss considerations moving forward on designing standard toxicity tests for scleractinian coral species. Factors include choice of test species and life-stage, specific test designs and replication requirements, inclusion of appropriate parameters for quality control (i.e. water quality, health assessments and appropriate negative and positive controls), timing of exposures, analytical verification, and finally the choice of appropriate biological endpoints for acute and chronic assessments to provide reliable and statistically appropriate measurements. The flow-through exposure system and methodology which was developed based on these principles was tested with two positive controls (copper and diuron) and UV filters in acute and chronic exposures. Analytical verification in each replicate confirmed that reliable and reproducible concentrations in expected ranges (based on nominal dosing) were achieved, water quality parameters remained within guidelines, and coral health was maintained throughout the 21-day duration for chronic tests.

#### **4.07.P Environmental Risk Assessment of Organic and Inorganic UV filters**

##### **4.07.P-Mo325 Experimental and Predicted Aquatic Ecotoxicity of Suncare and Rinse-off Products**

**Ludovic Faravel<sup>1</sup>, Pascal Bichere<sup>2</sup>, Floriane Larras<sup>2</sup>, Paul Thomas<sup>2</sup>, Laure Giamberini<sup>3</sup> and Carole Cossu-Leguille<sup>3</sup>,** (1)University of Lorraine, France, (2)KREATiS, France, (3)University of Lorraine, France



Over the last 10 years, cosmetic industry have grown to become one of the largest sectors using chemical substances in the world and is still growing. After use, residues of cosmetic products may end up in the aquatic environment despite the treatment of water in wastewater treatment plants. Consequently, they may represent a potential risk for organisms in the aquatic environment and their impact needs to be evaluated. The objective of this study was therefore to determine the aquatic ecotoxicity of cosmetic rinse-off and suncare products, to find out whether the products on the market or in development can present a risk for the aquatic environment. The results obtained will then help in the development of a predictive tool determining the toxicity of cosmetic products, which are mixtures of at least 15 different ingredients.

To meet these objectives, a battery of bioassays, where the ISO and OECD standards will be used for the various tests, was set up. Several species representative of different levels of the food web of the freshwater and marine aquatic environment were selected. These included primary producers: *Pseudokirchneriella subcapitata* and *Phaeodactylum tricornutum*, a primary consumer: *Daphnia magna*, a decomposer: *Aliivibrio fischeri* and secondary consumers: *Danio rerio* and *Hydra attenuata*. In addition to these tests, an OECD 301F biodegradability study modified to include analysis and supplementary parameters, will also be conducted to determine the extent of biodegradability of each finished product. These tests thus allow both acute and chronic toxicities as well as persistency of the tested cosmetic products to be assessed. Finally, *in silico* modelling studies are used to predict the toxicity of mixture and these are compared with the experimental data.

The results obtained showed that the most sensitive organisms are *P. subcapitata* and *A. fischeri* for the solar products and *P. tricornutum* for the rinse-off products. Despite this, the tested products show low toxicity to the aquatic environment, with EC50 values above 30 mg/L for acute tests and above 20 mg/L for chronic tests. These will be compared with iSafeRat® water accommodated fraction model developed by the ecotoxicologist of KREATiS. Further studies will be conducted and compared to various toxicity prediction models for mixtures to assess their validity.

#### **4.07.P-Mo326 Commercial Sunscreens Modulate the Gene Expression on *Physella acuta* Adults: Acute and Medium-term Exposure**

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The growing incidence of ultraviolet (UV) radiation has increased the concern and hence the use of sunscreens with special attention in spring and summer seasons. Therefore, UV filters (UVFs) presence in the environment has grown over the years, becoming one of the main emergent pollutants, mainly due to their inefficient removal in wastewater treatment plants (WWTPs). In this line, recreational activities such as sunbathing/swimming act as sources of UVFs to aquatic systems worldwide. The impacts of UVFs on aquatic organisms have been widely evaluated, mainly for marine environments, with single UVF exposure altering coral bleaching, physiological and biochemical responses on mussels or the endocrine system of fishes. However, these approaches are very far from real scenarios since sunscreens are composed of a mixture of different UVFs plus multiple chemical compounds for moisture, odour, etc. Therefore, in this work, three commercial sunscreens (SC1, SC2, SC3) were selected to study their impact on aquatic invertebrates, trying to mimic environmental conditions. Due to their lower flow, rivers tend to present higher concentrations of UVFs, partly due to the absence of tides. So, *Physella acuta*, an aquatic snail with a high capacity to live in adverse conditions and spread worldwide, was selected as a novelty organism for future toxicity test guidelines. The adults of *P. acuta* were exposed at 10 mg/L of each sunscreen for 7 days, and samples were collected at 24h and 7 days and frozen for the subsequent RNA extraction. The effects of the sunscreens were analyzed by Real-time PCR employing an array, including 42 genes related to essential metabolic pathways. The results showed a stronger response with downregulation after longer exposure, mainly in genes related to detoxification, redox, and stress response. Comparison between times showed mainly upregulation at 7 days of exposure respect to 24h, so *P. acuta* could need more time to respond concerning other aquatic invertebrates. There is a difference in the response to each commercial sunscreen, showing the lower toxicity in the case of SC1. To sum up, for the first time, the impact of commercial sunscreens on aquatic invertebrates at the sub-organismal level was evaluated. New biomarkers have been tested and proven their utility for future aquatic toxicology risk assessment. Furthermore, *P. acuta* has proven to be an effective sentinel organism for evaluating the effects of UVFs in freshwater systems.

#### **4.07.P-Mo327 Screening Thyroid Hormone Disruption of Several Organic UV Filters and their Mixtures with Rat Pituitary (GH3) Cells**

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Organic ultraviolet (UV) filters are suspected of adverse endocrine effects on humans and aquatic ecosystem. Avobenzone (AVB), diethylamino hydroxybenzoyl hexyl benzoate (DHHB), homosalate (HS), octocrylene (OC), octyl-methoxycinnamate (OMC, same as EHMC), and octisalate (OS) have been widely used as active ingredients in many sunscreen products, and have frequently been used in combination to enhance UV protection. However, their endocrine disruption, especially thyroid hormone (TH), is little understood. We screened thyroid disruption effects of major UV filters and their mixtures using rat pituitary (GH3) cells.

Six UV filters (AVB, DHHB, HS, OC, OMC, OS) were selected among frequently used organic filters. Then their mixture combinations were decided based on toxicity and usage pattern of the filters. Ten mixtures with 5 binary and 3 ternary combinations were chosen, which include combinations of UV filters with the same directions of thyroid hormone changes in zebrafish (*Danio rerio*), and another two binary combinations with high usage. After 48 hours of exposure, a NOAEL (no observed adverse effect level) of each UV filter was determined based on gene transcription in GH3 cells. Concentrations of

binary combinations were decided at x1/8, x1/4, x1/2, x1, x1.5, and x2 NOAEL, and those of ternary combinations were x1/27, x1/9, x1/6, x1/3, x1, x1.5, and x3 NOAEL.

After single exposure, organic UV filters significantly changed transcription levels of thyroid-relating genes in GH3, which included *Tshb*, *Trhr*, and *Dio2* genes. In mixtures, most of them showed similar effect directions — up-regulation of *Trhr* gene and down-regulation of *Tshb*, *Tra*, *Trb*, *Dio1*, and *Dio2* genes — although examples with opposite thyroid-disrupting responses *in vivo* were found. AVB+OMC, DHHB+OC, OC+OMC, AVB+DHHB+OMC, AVB+HS, and OMC+OS mixtures caused significant transcriptional changes at  $\leq$  x1 NOAELs, suggesting additivity or greater-than-additive effects.

In conclusion, the results from our study demonstrate that UV filters commonly used in marketed sunscreens may alter thyroid function either alone or in combination. Further studies are warranted to validate our observation and to understand the mixture effects of chemical exposure *in vivo*.

#### 4.07.P-Mo328 Presence and Environmental Risk Assessment of Organic UV Filters in Coastal Waters of the Iberian Peninsula

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Organic UV filters are increasingly used in different personal care products to protect against UV radiation. These substances have been receiving increasing attention due to their possible behaviour as endocrine disruptors and, therefore, could pose a risk to human health and the environment. They enter the aquatic environment mainly through bathing activities but can also be discharged from wastewater treatment plants and illegal dumping. Monitoring their presence in different environmental matrices, including coastal water/beaches, is essential to assess their exposure and potential risks and propose actions for their proper management. The objective of this study was to identify and quantify the presence of 21 UV filters in coastal areas of the Iberian Peninsula, which may be especially vulnerable during summer due to the high tourist pressure, to determine their environmental risk and detect “hot spots” where adverse effects on biota resulting from chronic exposure could be expected. Sixteen samples were collected along the Portuguese coast (Lisbon and Algarve) and 30 on the Spanish one: 18 in Cádiz and 12 in the Mar Menor (Murcia) in July and August 2021, during the peak tourism season. Sample analysis was performed using high-performance liquid chromatography coupled to tandem-mass spectrometry. Hazard was characterized using chronic toxicity data to calculate their Hazard Quotients (HQ). Measured concentrations and HQs were mapped to highlight Hot spots. Out of the 21 UV filters analysed, only 5 were found above the limit of detection: BP4, OC, BP3 and two of its metabolites BP1 and DHMB (BP8). BP4 was present in almost all samples (89%), followed by OC (35%) and BP3 (30%), while both metabolites were barely detected (3%). UV filters were detected in 43 sampling sites out of the 46 investigated. Samples collected in Mar Menor had the greatest variety of compounds per sample while the rest contained at least one of them. The highest total concentrations of UV filters were found in samples from Arcos beach (954 ng/L) in Lisbon, La Barrosa (607 ng/L), and Villanitos beach (1088 ng/L) in Mar Menor. Moderate (46%) and high (54%) risk was found for OC, while the others showed no or low risk, BP3 reached moderate risk in only 2%. No risk was estimated in any area for BP4 despite being the substance that appeared in the highest concentration in all the samples (633 ng/L). This study has been funded by Rey Juan Carlos University through “Bridge Projects”.

#### 4.07.P-Mo329 Development of Toxicity Test Protocols for Corals

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The risk assessment which is generally performed for any registration of chemical products did not cover corals in the past. First research on the effects of UV-filters started. However, for the generation of reliable and comparable toxicity endpoints it is essential to develop a standard toxicity test protocol. For risk assessment larvae or nubbins (branch tips consisting of several polyps) can be used. Beside acute testing also long-term tests using growth parameters can be performed under lab conditions. First studies showed that in case of extended test durations to 1-2 months, some coral species could be suitable to fulfil validity criteria according to OECD 239 (Water-Sediment *Myriophyllum spicatum* Toxicity Test) to generate robust and reproducible data. Endpoints could be assessed on growth rates and yields based on fresh weight, polyp number or total length of corals. We present data showing that tests with focus on coral growth parameters can be established and can assist in risk assessment of chemicals on corals.

#### 4.07.P-Mo330 Benzotriazole Ultraviolet Stabilizers Induce Toxicity in Early-Life Stage Fish Through Aryl Hydrocarbon Receptor Activation

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Benzotriazole ultraviolet stabilizers (BUVSs) help counter degradation and discoloration of plastic materials caused by UV radiation. Because BUVSs are ubiquitous in aquatic environments and biota, there is concern for the health of fishes. Studies suggest that certain BUVSs might cause toxicity by dysregulating the aryl hydrocarbon receptor (AhR) causing early life-stage toxicity in fishes. However, little is known about relative potencies of these chemicals as AhR agonists or about differences in sensitivity of phylogenetically diverse fishes to BUVSs that are AhR agonists. Therefore, the first objective of this study was to assess the toxicity of the three BUVSs - 2-(benzotriazol-2-yl)-4-methylphenol (UV-P), 2-(Benzotriazol-2-yl)-4-methyl-6-prop-2-

enyl-phenol (UV-9), and 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (UV-090) – using embryotoxicity assays with zebrafish (*Danio rerio*) and to assess the potency of each BUVS for activation for the zebrafish AhR2, using an *in vitro* luciferase reporter gene (LRG) assay with COS-7 cells transfected with the AhR2 of zebrafish. bryos exposed to BUVS by use of microinjection experienced mortality in a dose-dependent manner, with UV-P being the most potent BUVS with a median lethal dose (LD50) of 4772 ng/g-egg, which is less potent than TCDD, the prototypical AhR agonist. Compared to TCDD, relative potencies of UV-P, UV-9, and UV-090 were 0.0005, 0.0002, and 0.00004, respectively. The order of potency based on activation of the zebrafish AhR2 was UV-P > UV-9 > UV-090, which agrees with the embryotoxicity assay. The second objective was to assess species differences in sensitivity to AhR2 activation by each BUVS, by use of the *in vitro* AhR2 activation bioassay. There were interspecies differences in sensitivity to activation of the AhR by BUVSs. For UVP, the range of sensitivity was 6.4-fold, with zebrafish being the most sensitive species and brook trout being least sensitive. For UV9, the range of sensitivity was 2.6-fold, with zebrafish being most sensitive and lake sturgeon being least sensitive. UV-9 failed to activate the AhR for northern pike, white sturgeon, and brook trout. UV-090 did not activate the AhR in any species. This research gives valuable insight on the toxicity of BUVSs and can help guide objective assessment of risks posed by BUVS that have AhR agonistic properties.

#### 4.07.P-Mo331 Thyroid Hormone Disruption by Exposure to Mixtures of Major Organic UV Filters in Zebrafish (*Danio rerio*)

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Organic UV filters have been developed and applied to numerous products including cosmetics and sunscreens, to protect skin and products. Many sunscreens are manufactured with multiple UV filters in combination to maximize photoprotection of the products. Growing body of evidence supports negative impacts, including endocrine disruption, of organic UV filters. Nevertheless, little is known about the mixture interaction of UV filters. In the current study, avobenzene (AVB), diethylamino hydroxybenzoyl hexyl benzoate (DHBB), homosalate (HS), octocrylene (OC), octinoxate (OMC), and octisalate (OS) were chosen as target filters, and thyroid endocrine disrupting effects were evaluated for their mixtures using embryo-larval zebrafish (*Danio rerio*). First, no observed adverse effect level (NOAELs) were determined for each UV filter, based on thyroid hormone level (TH) changes following 5 day exposure after fertilization. Then, embryo-larval zebrafish were treated with binary or ternary combinations of a fixed-ratio dilution series, e.g., x1/8, x1/4, x1/2, x1, x1.5, x2 NOAEL. Thyroid hormone levels decreased following exposure to AVB+OMC (TSH, T3) and AVB+DHBB+OMC (TSH, T4, T3), at  $\geq 1 \times$ NOAEL. Exposure to OMC+OS significantly reduced TSH at  $\geq 1 \times$ NOAEL, and T3 even at  $< 1 \times$ NOAEL of OMC and OS, e.g., x1/8 NOAEL, demonstrating concentration additive effect on the thyroid system. Significant regulatory changes of *sult1 st5* or *ugt1ab* genes, which are responsible for TH metabolism and elimination, were observed by AVB+OMC, AVB+DHBB+OMC, and OMC+OS exposure, supporting the decreased thyroid hormone levels observed in the fish following exposure to the mixtures. Overall, our observation demonstrates that exposure to organic UV filter mixtures could impair normal thyroid function possibly through increasing hepatic THs elimination by *sult1 st5* and/or *ugt1ab* genes. Further studies on common mixtures of UV filters should be carried out.

#### 4.07.P-Mo332 A Reliable Method for Testing Acute Toxicity of UV Filters and Related Chemicals Towards Adult Corals

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Coral reef ecosystems are in decline due to global and local anthropogenic stressors. At coastlines with high touristic activity, the direct influx of organic and inorganic UV filters might be a significant local stressor for reef-building corals. However, the degree to which UV filters exhibit toxic effects towards corals is highly discussed and the use of various testing methods led to controversial results.

For this reason, we have developed a test method that can reliably assess the acute toxicity of UV filters and related chemicals to reef-building corals. The test is designed as a semi-static setup, has a duration of 96 hours, and includes a negative and positive control (CuCl<sub>2</sub>), plus a solvent control if necessary. The stony coral *Montipora digitata* was chosen as test species, since it exhibits adequate sensitivity to different chemicals, including UV-filters, and has a planar morphology allowing rapid creation of uniform discs (approx. 10 mm in diameter). The tests on coral fragments are carried out in artificial seawater to which different concentrations of the test substance have been added. To maintain the desired concentration of the chemical (> 80% of nominal), a water exchange is carried out after 48 hours. Accompanying analytics (ICP & UPLC-MS) are used to confirm exposure concentrations, and water parameters are measured to monitor water quality.

Endpoints indicating the toxicity of the tested chemical include bleaching, inhibition of photosynthetic efficiency and mortality / tissue loss. All endpoints are measured at the beginning and at the end of the experiment so that changes can be tracked on an individual basis. Predetermined validation criteria are used to evaluate the negative and positive control.

The testing system was successfully used with four different chemicals: Benzophenone-3 (BP-3), Copper-II-Chloride (CuCl<sub>2</sub>), 2,4-Dichlorophenoxyacetic acid (2,4-D) and Dimethylformamide (DMF), giving reproducible results which are presented.

The setup and workflow of the testing method was aligned with existing toxicological test protocols for other aquatic organisms (e.g., ISO 6341) and is reproducible in a standard laboratory setting. Therefore, the method can serve viable for ring testing and subsequent standardization under ISO, DIN and/or OECD guidelines.

#### 4.07.P-Mo333 Spatial Analysis on the Distribution of Sunscreen Use Patterns from Consumer Surveys Across the United States

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Information on consumer use patterns is an asset in understanding the qualitative and quantitative parameters of product usage across any geography. This is particularly relevant for consumer product use since such data is lacking, especially on a local scale to better understand the relevance from an environmental exposure perspective. Within consumer products, information on sunscreen usage is of special interest because of recent concerns about the toxicity of certain UV-filters. Sunscreen products contain chemicals that act as UV-filters, essential in preventing skin cancer by absorbing harmful UV radiation. However, there are concerns about the potential risk of some UV filter chemicals in aquatic environments. A survey across the United States (US) and Canada on sunscreen use by consumers provided a wealth of information on consumer usage behavior such as, application frequency, quantity of product, and swimming location (pools, lakes/ivers, oceans, etc.). Additionally, the survey data contained geographic relevance at a zip/postal code level, which is the most spatially detailed ever collected. Spatial analysis of the sunscreen use survey data provided insights into the per capita use, proximity to beaches and discharge to wastewater treatment plants – helping understand the environmental exposure to surface waters. Similarly, use quantity and direct exposure to ocean waters was examined. Further, the analysis also explored proximity of consumer use sources to exposure in aquatic environments. This spatially refined use information was extrapolated to adjust with national sunscreen use across the US and compared against wider data on temperature, cloud cover and UV index. The spatial and statistical analysis provided quantitative end points to correlate the relevance of sunscreen use to potential environmental risk. This research illustrates how consumer survey data about sunscreen habits and practices at a fine spatial scale can be used to better understand local environmental exposure concerns. The more refined understanding can better guide science-based risk management decisions for consumer products.

#### 4.07.P-Mo334 Occurrence and Potential Impacts of Organic UV Filters in *Acropora cervicornis* from the Florida Reef Tract

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Organic UV filters have been detected in seawater, typically in the ng to µg/L ranges, with some UV filters also found in biota highlighting uptake and a potential environmental risk from exposure. However, despite scientific and societal concern, there are a limited number of studies that have measured UV filter concentrations in seawater near coral reefs and even fewer that have examined the presence of UV filters in coral tissues. The studies demonstrating the presence of UV filters in coral tissues did not provide information on the health of the corals sampled or an overall health status of corals at the collection sites. Some laboratory exposure studies have suggested detrimental effects of UV filters on coral development and consequences to coral reproduction. This study is designed to address the knowledge gap between environmentally relevant UV filter exposures and effects on a key element of coral health, namely reproductive health. The levels of UV filters in matched seawater and coral tissue samples collected from various reef nursery sites in Florida was assessed. Correlations between the UV filter concentrations observed and reproductive health (fecundity) were made in the endangered staghorn coral (*Acropora cervicornis*), a keystone species in the Florida reef tract and throughout the Caribbean. This is the first study to provide data regarding UV filter concentrations in corals in the continental United States, as well as the first to examine the potential effects of environmental UV filter exposure on coral fecundity. The insights gained will provide critical support for regulatory policies and management decisions concerning UV filters, as well as create a foundation for future research in this new and emerging field of coral toxicology.

#### 4.07.V Environmental Risk Assessment of Organic and Inorganic UV filters

##### 4.07.V-01 Aquatic Risk Assessment of Product-Released Engineered Nanomaterials (PR-ENMS) From Personal Care Products

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Majority of studies thus far have used pristine engineered nanomaterials (ENMs) or in silico approaches to establish the environmental nanotechnology risks, approaches which may over- or under-estimate the risk of product-released ENMs (PR-ENMs). This study determined the environmental exposure of titanium dioxide and zinc oxide nanoparticles (nTiO<sub>2</sub>, nZnO) PR-ENMs and their toxicity to aquatic organisms due to their high application as UV filters in personal care products. The inorganic UV filters were released from products using environmentally realistic methods and characterised with a suite of physico-chemical analytical techniques. Toxicity was assessed using *Daphnia magna* (acute immobilisation test and reproduction test), *Raphidocelis subcapitata* (growth inhibition test) and *Danio rerio* (fish embryo tests); organisms chosen to represent micro-, macro-invertebrates and fish communities. Considerable release of nTiO<sub>2</sub> (from sunscreen), and nZnO (from baby cream) from the products occurred, additionally, nZnO released Zn<sup>2+</sup> ions. The released ENMs were needle-shaped (nTiO<sub>2</sub>) and angularly shaped (nZnO), with the sizes of 33.1±1.2×11.6±0.5 and 109.6±6.6×55.7±3.9 for nTiO<sub>2</sub> and nZnO respectively. The toxicity was prevalent on all the tested organisms with lower order organisms (microorganisms) exhibiting relatively higher toxicity than higher order organisms (fish). PR-nTiO<sub>2</sub> was growth inhibitive to microalgae by 80% at the highest concentrations while the

effects were mild (average 50% immobilisation) on *Daphnia magna*. PR-nZnO and Zn<sup>2+</sup> induced higher toxic effects on *D. magna* (EC<sub>50</sub>s were 10.47% and 14.63% for nZnO and Zn<sup>2+</sup>, respectively) relative to PR-nTiO<sub>2</sub>. The same was true for *R. subcapitata*. The effects of both UV filters were mild on the survival of fish embryo but comparable in inducing morphological malformations in the embryo. The results point to considerable risk to the aquatic organisms from PR-ENMs' environmental exposure. Thus, the determination of environmental exposure and effects in actual aquatic environments is required in reducing the probable risks of PR-ENMs. Now, more than ever, risk mitigation should be the integral part of product development and manufacturing.

#### 4.08 Exposure and Effect Assessment of Ionic and Ionizable Organic Chemicals

##### 4.08.T-01 Hazard and Fate Assessment of Permanently Charged Compounds

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Most of studies concerning Ionic and Ionisable Organic Compounds (IOCs) pertain to acids/bases or their simple inorganic salts (halides or alkali metals), where in fact only one entity needs to be considered (the other being H<sup>+</sup> or Na<sup>+</sup>, K<sup>+</sup>, C<sup>-</sup>, I<sup>-</sup> etc.). Many pharmaceuticals, surfactants or PFAS are such examples. There is however a broad group of substances – known as ionic liquids (ILs) – where both the anion and the cation can be of environmental relevance. We looked into most important issues in environmental impact assessment of such substances using the example of ILs which can be used in crop protection as so called Systemic Acquires Resistance inducers (SAR inducers).

As expected, transforming a neutral compound into its simple ionic derivative (acid or K-salt) has mitigating effect on toxicity, while replacing the counterion with more hydrophobic entity vastly increases toxic potency. Within the set of investigated structural analogues EC<sub>50</sub> values spanned over five orders of magnitude.

In assessment of ready biodegradability (most widely used for screening-level assessment) high biodegradability levels can be obtained even if one of the ions is persistent depending on the distribution of carbon between the ions. We suggest that in case of ILs, if anion and cation are amenable to biodegradation, they should be assessed both together and separately in the form of inorganic salt of the anion and the cation. The extent of biodegradation of both entities should also be assessed using specific analytical method.

Lack of experimental data does not allow for assessing the accuracy of K<sub>oc</sub> predictions by COSMO-RS for tested ILs. HPLC method for screening log K<sub>oc</sub> values of ILs was shown to deliver variable results so that validation using higher tier data is necessary.

In a broader context the question arises of how ILs will behave when released in the environment and what are the implications for hazard assessment. We have shown that both ions contribute to toxicity and biodegradability. Additionally, one ion can mask lack of the biodegradability of the other if test based on sum parameters is used without analytical verification.

We expect ILs to behave like separate ions in the environmental where other ions are abundant. In the same time, we believe that explicit attention should be paid to both the anion and the cation. Moreover, many experimental methods have too narrow a range to handle ILs or lack experimental verification.

##### 4.08.T-02 Assessment of Methods for Determining the Membrane-water Partition Ratio for Surfactants

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Surfactants are often classified as difficult to test chemicals (Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures), which may lead to uncertainty in chemical assessment. A key factor hampering adequate chemical fate modelling is that octanol-water partition coefficients (K<sub>ow</sub>) are often highly uncertain for surfactants, and poorly representative of the interaction energy of ionic surfactants with soil and tissue matrices. The phospholipid membrane-water partition ratio (K<sub>mw</sub>) is a promising alternative chemical descriptor for surfactants instead of K<sub>ow</sub>, as it relates to partitioning into a critical cellular component and accounts for ionic interactions. K<sub>mw</sub> can be determined by several experimental methods using artificial phospholipid material as unilamellar liposomes, bilayer coated silica, HPLC columns, or cells. Consistent K<sub>mw</sub> values have been measured for a variety of surfactants. Also promising computational approaches to calculate K<sub>mw</sub> have been evaluated. The overall aim of the study is for a systematic evaluation of three experimental methods and three computational approaches to determine K<sub>mw</sub> for a diverse set of surfactants, covering nonionic, anionic, cationic, and zwitterionic structures. Based on this assessment, we aim to further establish confidence in the K<sub>mw</sub> approach for different types of surfactants, provide guidance on the most cost-effective assay for specific surfactant classes, calibrate computation approaches, and make a next step towards standardization of validated test protocols. Here we present the initial phase of the work comparing experimental methods and molecular dynamics simulations for nonionic and anionic surfactants, using HPLC columns with Immobilized Artificial Membrane coated silica (IAM-HPLC), Solid-supported lipid membranes (SSLM), and unilamellar liposomes in dialysis systems. The data set provides relevant data to derive first tier estimates on bioaccumulation, and most likely also on baseline toxic potential.

#### 4.08.T-03 A Food Web Bioaccumulation Model for Pharmaceuticals in Aquatic Ecosystems

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With increasing medical and veterinary use, pharmaceuticals have been detected as emerging pollutants in global water bodies. The continuous inflow of pharmaceuticals into the environment potentially imposes adverse effects on aquatic ecosystems and human health. Food web models in aquatic ecosystems (water-algae-daphnid-fish-bird/mammal) are useful tools to estimate potential exposure across trophic levels to pharmaceuticals. However, many pharmaceuticals are ionisable, and food web models accounting for ionisation are rare. Therefore, the present study aimed to develop a food web bioaccumulation model for pharmaceuticals in aquatic ecosystems and evaluate its performance.

The model was established based on the OMEGA bioaccumulation model, combining both mass balance and allometric theory to predict the bioaccumulation of chemicals throughout the food chain. Default values for parameters are obtained as functions of chemical properties (octanol-water distribution coefficient;  $D_{ow}$ ) and biological traits (body mass). The sorption capacity of the organism for ionised pharmaceuticals compared to water ( $K_{BW}$ ) was estimated through existing polyparameter linear free energy relationships (pp-LFERs), taking electrostatic effects into account. Model performance was subsequently evaluated by comparing estimated pharmaceutical concentrations in different trophic levels with measurements.

Two sets of results were computed. With  $K_{BW}$ s related to octanol as a function of  $D_{ow}$ , concentrations in organisms were lower than those estimated based on pp-LFERs. The difference was mainly attributed to the partitioning of acids (negatively charged) to serum albumin (positively charged lysyl- or arginyl residues) and the partitioning of bases (positively charged) to phospholipids (negatively charged phosphate). The model tends to underestimate concentrations of basic pharmaceuticals in algae. This may indicate that other processes such as ion-trapping and/or adsorption on cellulose surfaces may be important for basic pharmaceuticals, which are currently not included in the model. The present study is the first to develop a food web accumulation model that explicitly accounts for ionisation. The developed model will ultimately estimate the accumulation of pharmaceuticals in birds and mammals. Therefore, it will be possible to screen pharmaceuticals in terms of their potential to cause secondary poisoning.

#### 4.08.T-04 Disentangling the Mechanisms Driving Accumulation and Elimination of Perfluoroalkyl Acids (PFAA) Using Toxicokinetic Modeling in Knock-out Mice

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Perfluoroalkyl acids (PFAA) are the most studied chemicals within the broad family of per- and polyfluoroalkyl substances (PFAS) and have been detected in virtually all humans. Elevated PFAA concentrations in specific tissues such as the liver have been linked to adverse human health outcomes such as non-alcoholic fatty liver disease. However, mechanistic studies investigating the importance of specific tissue binding, transport and reabsorption mechanisms, and excretion for PFAA are scarce, resulting in a limited ability to constrain physiologically-based toxicokinetic (PBTK) models that could simulate tissue-specific accumulation and disease risks. Here, we develop and evaluate a multi-compartment PBTK model using temporal concentrations of PFAA measured in blood, excreta, and tissues sampled from mice after a single intravenous administration. The importance of albumin, fatty-acid binding proteins (FABPs), and organic-acid transporter proteins (OATPs) to internal PFAA distribution is evaluated using mice with specific biomolecules knocked out. The PBTK model accounts for: (1) blood flow to and from organs; (2) binding to tissue components (e.g. albumin, FABPs); (3) passive diffusion through membranes (permeability); (4) active transport by membrane transporters (e.g., OATPs); (5) enterohepatic circulation and tubular reabsorption; and (6) excretion via feces and urine. Chemical transport data are derived from *in vitro* cell assays and partitioning experiments and physiological model parameters are based on literature data. Preliminary simulations suggest unbound PFAA in all tissues to decrease with increasing carbon chain length (hydrophobicity). Retarded elimination is supported by strong binding to albumin and phospholipids and reabsorption from the liver and kidneys back into the bloodstream. Simulations that include active transport mediated by OATPs can explain higher tissue: blood ratios compared to those based only on membrane permeability. Further feedback loops between model simulations under varying input parameters and elimination experiments with knock-out mice will gradually identify the mechanisms driving PFAA accumulation and elimination in mammals. The findings will prioritize processes to be investigated in humans to clarify the link between the internal distribution and adverse health effects of PFAS.

#### 4.08.T-05 Role of Bioavailability and Protein Binding of Perfluoroalkyl Substances in Cell-based Bioassays for Quantitative In Vitro to In Vivo Extrapolations

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Ubiquitous per- and polyfluoroalkyl substances (PFAS) pose a risk to human health. New approach methods (NAM) support risk assessment of PFAS but require quantitative *in vitro* to *in vivo* extrapolation (QIVIVE). The quantification of freely dissolved concentration ( $C_{free}$ ) is a bottleneck of QIVIVE, since PFAS not only partition to lipid but also bind to protein, thereby reducing the bioavailability of PFAS, which results in  $C_{free}$  being lower than the nominal concentration ( $C_{nom}$ ).

A method based on C18-coated solid phase microextraction (C18-SPME) fibers was developed to study the binding of four anionic PFAS (perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorobutanoate (PFBA), perfluorohexane sulfonate (PFHxS)) to bovine serum albumin (BSA) as a function of concentration, as well as their partition constants to medium,

cells and blood plasma. These parameters were used to predict  $C_{\text{free}}$  of PFAS in both cell bioassay and plasma by a concentration-dependent mass balance model (MBM). Anionic PFAS were found to bind to BSA in a concentration-dependent manner, including specific and non-specific binding.  $\log K_{\text{specific}}$  were derived from the specific saturable binding of PFAS to BSA and used to calculate the distribution ratio between plasma and water ( $D_{\text{plasma/w}}$ ) by MBM with the volume fractions of protein ( $VF_{\text{prot}}$ ) and lipid ( $VF_{\text{lip}}$ ) in plasma.

As exemplary *in vitro* bioassay, a reporter gene assay indicating activation of the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ -GeneBLazer) was chosen, and blood plasma levels were collected from literature for occupational exposure and the general population. A simple approach for QIVIVE is the ratio of concentrations ( $C_{\text{nom}}$  or  $C_{\text{free}}$ ) in human blood to that in the bioassays. The QIVIVE $_{\text{nom}}$  ratios were of concern for PFOS (22.7) and PFOA (1.96) for occupational exposure and were up to thousands of times higher than the QIVIVE $_{\text{free}}$  ratios due to the strong binding affinity to proteins and large differences in protein contents between human blood and bioassays.

This study demonstrated that C18-SPME is a versatile method to quantify  $C_{\text{free}}$  and partition constants of PFAS under highly diverse conditions and over many orders of magnitude in concentrations. MBM can be used to estimate the  $C_{\text{free}}$  for more high-throughput *in vitro* assays from Tox21 database if more partition constants of PFAS are available in the near future.

#### 4.08.P Exposure and Effect Assessment of Ionic and Ionizable Organic Chemicals

##### 4.08.P-Mo335 Investigating the Role of Biotransformation in the Toxicity of Ionizable Organic Compounds, Using LC-HRMS – Zebrafish Embryos Exposed to Ibuprofen as a Case Study

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Pharmaceuticals as well as their transformation products are highly detected in the aquatic environment and may bioaccumulate in aquatic organisms causing adverse effects. Ibuprofen (IBU) is a commonly used nonsteroidal anti-inflammatory (NSAID) drug which has been reported in aquatic environments. Many pharmaceuticals belong to the group of ionizable organic chemicals (IOCs) and their properties appear to be affected by the pH of the surrounding medium. However, the influence of pH values on the toxicity of IOCs hasn't been extensively evaluated in risk assessment studies.

The zebrafish embryo (ZFE) has emerged as a powerful alternative model organism, which is widely used in ecotoxicological research studies for evaluating the potential effects of xenobiotics on aquatic organisms.

The scope of this study was to assess the influence of the different pH values of the test medium on the uptake, bioaccumulation, biotransformation as well as toxicity of IBU in ZFE. Another goal was to identify the potential bio-TPs of IBU and to determine their concentration. Our overall objective was to investigate whether using biotransformation data complementary to the internal concentration ( $C_{\text{int}}$ ) of the parent compound, could provide a holistic toxicity interpretation at different pH values of the exposure medium.

Fish embryo toxicity test (FET) with ZFE was conducted at three pH values and the LC<sub>50</sub> values of IBU were determined. For the extraction of IBU in ZFE samples, organic solvents were added, and a bead-beating homogenization process was followed. The extracts were analyzed by RPLC and HILIC, in both positive and negative ionization modes, using LC-HRMS. A target-screening approach was followed for the identification of IBU, whereas identification of tentative bio-TPs was performed through in-house developed suspect and non-target screening workflows.

The pH-dependent toxicity of Ibuprofen was investigated, highlighting the importance of including the pH as a factor in toxicity studies. Concerning biotransformation, more than 10 bio-TPs were identified and a potential biotransformation pathway of IBU was proposed. It turned out that biotransformation greatly contributes to the  $C_{\text{int}}$  of the parent compound and may affect its toxicity. Therefore, biotransformation should be thoroughly investigated in toxicokinetic studies, complementary to  $C_{\text{int}}$ , for a holistic interpretation of the toxicity of IOCs.

##### 4.08.P-Mo336 The Use Of Toxicokinetic Models To Improve The Understanding Of Internal Concentration For Ionisable Organic Chemicals In Fish

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Regulatory frameworks for assessing chemical safety have had historically a reliance on *in vivo* testing. However, the global regulatory landscape is experiencing an evolution in thinking around the use of animal testing. Hence, data requirements are also changing towards the use of more New Approach Methodologies (NAMs) based on mechanistic knowledge from *in silico*, computation and *in vitro* sources. This trend towards the use of *in vitro* based NAMs in safety decisions is dependent on the availability of robust Physiologically Based Kinetic (PBK) models in order to support a better understanding of the relationship between the internal and external concentrations of chemicals through *in vitro* to *in vivo* extrapolations (IVIVE). To enable this, there is a compelling need to develop and build confidence in *in-silico* models such as toxicokinetic (TK) models that can be used to support the risk assessment of these chemicals.

While TK models are already available, they are focussed mainly on neutral organic chemicals. Yet, there are a large number of chemicals which have the potential to ionise in the aquatic environment and their potential impacts on organisms are often poorly characterised. Hence, TK models specifically designed for ionisable chemicals are needed to allow for the prediction of the internal concentration that is likely to elicit toxicity in the organism. In this study, we evaluate the potential applicability of a one-

compartment TK model for fish species, where physicochemical descriptors such as the  $pK_a$  and  $D_{ow}$  (octanol-water partition coefficient corrected for the pH) alongside with species traits such as the lipid fraction, could improve its performance and result in a better prediction of the relationship between the internal and external concentrations in fish species for ionisable compounds. This study will build on existing TK models by bringing more realism and expanding their applicability domain including ionisation. Furthermore, it will support the understanding of the mechanisms of uptake and elimination in fish and the main drivers underpinning them.

#### **4.08.P-Mo337 Solid-Phase Microextraction Elucidates the Binding of Perfluoroalkyl Acids (PFAA) to Surrogate Biomolecules Relevant to Their Distribution in Humans**

*Sophia Kozart Ludtke, Fabian Christoph Fischer and Elsie Sunderland, Harvard University*

Perfluoroalkyl acids (PFAA) are anthropogenic chemicals that have been associated with adverse human health effects like liver and kidney diseases following exposure to contaminated drinking water, food, and other sources. Despite their importance, factors controlling PFAA accumulation in human tissues and interactions with biomolecules (e.g., proteins, lipids) are still poorly understood. Binding affinities to biomolecules can be quantitatively described by empirically measured partition coefficients. This presentation will report newly measured partition coefficients for PFAA with varying carbon chain lengths (C3-C11) and functional groups (e.g., carboxylic and sulfonic acids) to biomolecules and biofluids suspected to be drivers of tissue accumulation in humans using a solid-phase microextraction (SPME) method with C18-coated fibers. Factors experimentally tested at chemical concentrations ranging from typical human exposure levels to high doses administered in mammalian toxicity tests include concentrations of human serum albumin (HSA), fatty-acid binding proteins (FABPs), bile, globulin, and casein. Preliminary fiber-water experiments showed high reproducibility and linear binding over the tested concentration range, with equilibrium achieved in < 48 hours for all tested PFAA. PFAA with longer carbon chains (greater hydrophobicity) were generally associated with higher binding to biomolecules. Perfluorinated sulfonates sorbed more strongly to proteins than their corresponding carboxylic acids. Sorption to bile showed the opposite trend, suggesting that enterohepatic circulation might be of higher relevance for perfluorinated carboxylic acids. These partition coefficients can broadly inform physiologically-based toxicokinetic (PBTK) models to help advance understanding of the driving binding mechanisms for organ-level PFAA distribution and accumulation. As a next step, the SPME method will be applied to compare the binding of PFAA to human tissues (e.g., liver, kidneys) sampled in Cohort studies to “model tissues” constructed as combinations of surrogate biomolecules and their respective partition coefficients.

#### **4.08.P-Mo338 Developing Mass Balance Models for Simulating Indoor Fate and Human Exposure to Ionic and Ionizable Organic Chemicals Released Indoors**

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People spend most of their time in indoor environments where they are exposed to a wide array of neutral, ionic, and ionizable organic chemicals (IOCs). Humans directly ingest, inhale, or apply chemicals to their bodies in the form of pharmaceuticals and consumer and personal care products (PCPs). Chemicals released indoors can accumulate in dust and on surfaces and volatilize in indoor air. Humans subsequently encounter these chemicals indirectly through inhalation, indirect dermal contact, and mouthing-mediated (i.e., hand-to-mouth or object-to-mouth) ingestion of dust or residues. Mass balance chemical fate models are important tools for human health assessments and for elucidating, quantifying, and comparing pathways of exposure. Such models have been developed, applied, and evaluated for several classes of neutral organics for which intermedia partitioning behaviour can usually be well characterized. However, many organic chemicals used in indoor environments are ionic or ionizable, and the charged species of such chemicals may behave differently from their neutral forms. The RAIDAR-ICE model combines an indoor multimedia mass balance chemical fate model with a human exposure and physiologically-based biokinetic (PBK) model. This integrated and holistic approach enables modeling human external and internal exposures to chemicals in various indoor compartments (e.g., air, dust, and indoor surfaces) through multiple near-field exposure routes (e.g., inhalation, ingestion, and dermal exposure). RAIDAR-ICE has been revised to more explicitly quantify the indoor fate and transport of and exposure to IOCs. Partitioning of IOCs is calculated using distribution ratios, and indoor media are characterized as containing both organic and aqueous components. Here, the RAIDAR-ICE model is applied in a case study for consumer and occupational exposures to select IOCs. The calculated dust and air concentrations, as well as human intake rates and biological concentrations are compared to monitoring data (where available) and the model performance is discussed. The relative importance of explicitly considering dissociation for IOCs in human exposure estimation is highlighted.

#### **4.08.P-Mo339 Toxicological Properties of Imidazolium-Based Surface-Active Ionic Liquids (Im-SAILS); A Case Study using the Brackish Water Shrimp, *Palaemonetes africanus***

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Imidazolium-based surface-active ionic liquids (Im-SAILS) are cationic surfactants having long hydrophobic alkyl chains. They exhibit unique aggregation behaviour and are being explored for use in oil recovery and other biotechnological applications. This class of surfactants are believed to be environmentally friendly with low toxicity. However, studies to assess the toxicity of SAILS have been largely short-term acute toxicity studies using microorganisms. In the present study, we investigated the acute and sublethal toxicity of three Im-SAILS to a macroinvertebrate, *Palaemonetes africanus*, using laboratory bioassays. The shrimp is a brackish water shrimp able to tolerate varying salinity conditions. Toxicological tests were conducted using reconstituted water with a salinity of 5‰. The 96hr LC<sub>50</sub> acute toxicity index of the three Im-SAILS assessed; 1-methyl-3-octylimidazolium



tetrafluoroborate, 1-Decyl-3-methylimidazolium tetrafluoroborate, and 1-dodecyl-3-methylimidazolium tetrafluoroborate against the species were 18.52 mg/l, 18.17 mg/l and 3.93 mg/l respectively. The sublethal effects of the Im-SAILS on the shrimps were assessed by determining the levels of oxidative stress enzymes and substrates of oxidative stress after a 14-day exposure to sublethal concentrations. The results showed that the Im-SAILS did not induce sublethal toxic effects in the exposed shrimps. Results from the present study have established that Im-SAILS can be acutely toxic to macroinvertebrates. This indicates a need to regulate the application of Im-SAILS in the biotechnological process and maintain environmental concentrations below toxic levels. These can be achieved through regulatory testing and the establishment of environmentally safe limits for Im-SAILS.

#### 4.08.P-Mo340 Why pH Matters in Cell-based in vitro Bioassays

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The speciation of ionizable organic chemicals (IOCs) is dependent on the pH-value of their environment. As different chemical species may exhibit different degree of toxicity towards living cells or whole organisms, the toxicity of IOCs is typically pH-dependent. In this study we measured the pH-dependence of cytotoxicity and activation of oxidative stress response for 11 (7 basic, 1 acidic and 3 multifunctional) IOCs with at least one acidity constant ( $pK_a$ ) between 6.5 and 9. The  $pK_a$  values of all test chemicals were quantified using UV-metric or potentiometric titration. For the toxicity tests the AREC32 reporter gene assay based on genetically modified MCF7 cells was used. In general, cytotoxicity increased with increasing fraction of neutral or net-neutral (zwitterionic) species, which is in line with previously published data for organisms like daphnia and algae. The basic IOCs showed increasing cytotoxicity and activation of oxidative stress response with increasing pH, while toxicity slightly increased with decreasing pH-value of the medium for the acidic chemical benzothiazolone. For the multifunctional chemicals labetalol and cetirizine toxicity increased with increasing fraction of the zwitterionic species. The activation of oxidative stress response showed less dependence on the pH value of the medium for the majority of the test chemicals. For labetalol the measured cytotoxicity could be explained by baseline toxicity and did therefore correlate with the liposome-water distribution ratio  $D_{lip/w}$  (pH). For all other test chemicals reliable  $D_{lip/w}$  (pH) were missing and no in-depth analysis was possible. The diprotic base 1H-Imidazole-1-propanamine showed decreasing cytotoxicity and activation of oxidative stress response with decreasing fraction of the dicationic species. This effect cannot be explained by baseline toxicity as the dication is expected to have much lower membrane affinity compared to the cationic and neutral species. This also indicates that pH-dependence of toxicity in cell-based bioassays may not easily be predictable by QSAR models.

#### 4.09.A Mechanistic Effect Modelling for Regulatory Environmental Risk Assessment: From Molecules to Landscapes

##### 4.09.A.T-01 Compound-specific Toxicokinetics and Physiological Response Patterns of two Target and one Non-Target species

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Insecticides play an important role in preventing or reducing insect damage to crops and ensuring yield quality and quantity of crops. Targeted pest species, as well as non-target species, live in agricultural ecosystems and insecticide research and development aim to optimize selectivity. Physiological traits, such as feeding behaviour and biotransformation, can influence the toxicokinetics (TK) and thus present a lever to optimize species selectivity. It is unknown how target and non-target species differ in these physiological traits. TK of insecticides need to be understood independently of the toxicodynamics, i.e., insecticidal effects.

Five test compounds were investigated that range in lipophilicity (LogP 1.43-3.57) and are made up of scaffolds or fragments from known insecticides yet being insecticidally inactive. Single *Spodoptera littoralis* (pest) larvae were exposed to test compounds on soybean leaf discs by feeding and surface contact. Food consumption and transformation into faeces were recorded by image analysis. *Myzus persicae* (pest) mixed age populations were exposed by oral uptake in a sachet feeding assay. Continuous feeding was captured by honeydew excretion. *Chironomus riparius* (non-target) larvae were exposed actively and passively in water solutions (OECD guideline). Body residues, and excreted compounds were quantified as a function of time during the exposure and a follow-up depuration phase. Parent compounds and putative metabolites were separated by liquid chromatography and detected by mass spectrometry. A TK-model was built and parameterised to quantify uptake, biotransformation, and elimination rate constants. The exposure did not disturb continuous food consumption, nor excretion of exposed *Spodoptera* larvae and *Myzus* population in experiments. No disturbance in movement of exposed *Chironomus* larvae was observed. Body residues differed significantly between species and across compounds, quantified by differences in uptake, biotransformation, and elimination rate constants.

The bespoke bioassays enabled the measurement of body residues in target and non-target species to build species TK models. Knowledge about TK across these species could be used to achieve maximum effectiveness against pests at minimum environmental impact. Thus, this new knowledge on selectivity constitutes an important building block for sustainable food production and environmental safety.

#### 4.09.A.T-02 Physiologically Based Gonadotropic Axis Kinetic Model in Female Zebrafish (*Danio Rerio*) Exposed to Two Azole Fungicides (Prochloraz and Imazalil)

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The reproductive function of vertebrates relies on the delicate dialogue between the gonads and the hypothalamus-pituitary complex defining the gonadotropic axis (i.e. hypothalamic-pituitary-gonadal axis, HPG axis). Its disruption by endocrine disruptors (ED) is well known and focuses mainly on several endpoints leading to an alteration of reproductive and developmental functions. Therefore, predicting the bioaccumulation of an ED is a key point to understand its underlying mechanism with the HPG axis. Physiologically based toxicokinetic (PBTK) models are useful tools to easily predict the bioaccumulation of a substance in an organism and enable a mechanistic approach.

Within the GinFiz project (APR EST ANSES n  2020/01/133), zebrafish was selected to study the alteration of the HPG axis. Also, two azole fungicides: prochloraz (PCZ) and imazalil (IMZ), were chosen for their well-known ED properties. Thus, this work aims to elucidate the kinetic profiles of PCZ and IMZ in the fish to understand their bioaccumulation and to translate their toxic effects into the HPG axis in the fish. To start with, the PBTK model predicted every data point available (i.e. internal concentrations in several fish organs) within a 3-fold range and 92% within a 2-fold range. Thereafter, the HPG axis was successfully integrated into the PBPK model and enabled the prediction of the physiological kinetics of steroid hormones, luteinizing hormones, and vitellogenin. This model will allow the investigation of the toxic effect of these two azole fungicides on the HPG axis in female zebrafish, such as steroid hormone balance.

#### 4.09.A.T-03 Is Considering a Physiologically Based Toxicokinetic Module in a GUTS Model Really an Added Value or Not?

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In the current Anthropocene, environmental pollution is a global problem that is inextricably linked with rapid industrialization and urbanization. Pollution hampers the environment sustainability and ecosystem services. The decline in environmental quality, because of pollution, is evidenced by a loss of vegetation, a loss of biological diversity, and excessive amounts of harmful chemicals in all compartments of the biosphere. As a result, the impact of chemicals on human health is today recognized as coming from contaminated air, soil, and water, via direct (e.g., ingestion) or indirect (trophic chain) exposure routes. Therefore, a holistic approach is an urgent need to remove the pollutants in a drastic way. Preserve the survival of living organisms in the face of chemical contamination is today among the biggest challenges of regulatory bodies, pollution and contamination of natural resources adversely affecting livelihood of all species. Nevertheless, could Europe really move towards zero pollution, in line with the ambition of the European Green Deal? If it is now several decades since concentration-response or effect models are used to estimate x% lethal or effective concentrations (LCx or ECx), mechanistic effect models (MEMs) were recently recognized of high potential to make risk assessment of chemicals more ecologically relevant than current standard practices [1]. MEMs consider the underlying mechanisms of how chemicals affect individual life history traits, linking the exposure concentration to the internal damages it causes (toxicokinetic), that in turn translate into a reduced fitness (toxicodynamic). MEMs belong to the so-called toxicokinetic-toxicodynamic (TKTD) models. Among TKTD models, the one dealing with survival, entitled GUTS for General Unified Threshold model of Survival [3] is already considered as ready-to-use for environmental risk assessment by regulatory bodies [3]. Nevertheless, the TK part of GUTS is oversimplified considering organisms as a whole and characterizing uptake and elimination by one single parameter  $k_d$  (dominant rate constant). Based on recent advances in the development of physiologically based toxicokinetic models [4], we present here an innovative extended GUTS model including a multi-compartment toxicokinetic model. This physiologically based TKTD model (namely PBGUTS) will be presented in this talk from a case where gammarids were exposed to zinc and cadmium environmental concentrations.

#### 4.09.A.T-04 Physiologically-Based Kinetic Models for Bird Species and Application to Fluopyram

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Physiologically-based kinetic (PBK) models are effective tools for designing toxicological studies and conducting extrapolations to inform hazard characterization in risk assessment by filling data gaps and defining safe levels of chemicals. Toxicity testing in birds is performed as a requirement for pesticide registration by regulatory authorities (Regulation (EC) No 1107/2009), and PBK models have the potential to interpret such data mechanistically and to extrapolate to different exposure scenarios. PBK models represent the body as a network of interconnected compartments linked via blood flow. After an oral, dermal, or inhalation exposure to a compound, its absorption, distribution, metabolism, and excretion processes are simulated by means of ordinary differential equations, allowing to simulate time-concentration curves in target organs.

The aim of the present work was to develop a generic whole-body PBK model for male and female birds, for three bird species, chicken (*Gallus gallus*), duck (*Mallard duck*), and bobwhite quail (*Colinus virginianus*). The reproductive system includes ovary, oviduct, egg white and yolk compartments to predict detailed concentrations in eggs. For the model development several partitioning coefficient (PC) calculation methods were explored. The developed PBK models were then qualified (validated) against available *in vivo* data. Finally, models were documented and scored following international guidance documents from WHO, EFSA, and OECD.

Simulations of tissue time-concentration (PK profile) curves were run for the ten chemicals chloramphenicol, deltamethrin, florfenicol, ivermectin, melamine, midazolam, monensin, itraconazole, salinomycin, and fluopyram (with metabolite fluopyram-benzamide). Insights into the outcome for the fluopyram use case chemical will be provided in the presentation, where multiple exposure scenarios were evaluated on their impact on egg concentrations.

In order to assess the performance of the models, an analysis of the overall data points against the model predictions as a “goodness of fit” criterion was done. The models were evaluated within a range of -three and -tenfold to account for variability from the *in vivo* studies. The performance analysis was conducted for each chemical by comparing all observed *in vivo* data by species. The overall accuracy of the model predictions across the chemicals analyzed was found to be species- and compound-specific.

#### 4.09.A.T-05 Reproductive Toxicity in Birds Predicted by DEB-TKTD Modelling

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Toxicokinetic-toxicodynamic models based on dynamic energy budget theory (DEB-TKTD models) can predict sublethal toxic effects at the organism level. However, relatively few studies have used DEB-TKTD models to predict impacts of pesticides on bird and mammal species. This lack of case studies was highlighted as a key obstacle to the use of DEB-TKTD models in ERA by the recent draft EFSA guidance document on ERA for Birds and Mammals. We used DEB-TKTD modelling to mechanistically predict the effects of fluopyram on the weight of bobwhite quail (*C. virginianus*) hatchlings and 14-day old chicks following parental exposure via the diet. We assumed that some of the chemical is transferred from the mother into eggs. The model was calibrated to a novel experiment in which eggs were injected with benzamide (the metabolite of fluopyram found in a study on laying hens) at various concentrations. Effects on embryo growth, survival, and yolk utilization were measured during incubation. Model calibration tested three physiological modes of action (pMoAs) – stress on maintenance costs, growth costs, or reserve mobilization – as well as two TK assumptions: that benzamide exposure of the embryo begins upon injection or starts with yolk absorption on day eight. The experimental data showed reduced embryo growth as well as reduced yolk utilisation with increasing benzamide concentration. The choice of pMoA was thus clear, as this pattern could only be matched by selecting stress on reserve mobilization. The fitted model closely matched data for all endpoints. It was then used to predict effects on hatchling weight and 14-day chick weight (normalised to percentage reduction vs controls) in an independent dataset, in which bobwhite quail females were exposed to fluopyram via diet, and hatchling and chick weights were monitored (OECD 206 study). To infer benzamide concentration in the egg resulting from parental exposure we used a physiologically-based TK model. Data were well matched by predictions, with 87.5% of all data points predicted to within one standard deviation. The results show that DEB-TKTD models calibrated to embryo data can predict effects at hatching and at later life-stages. This study also demonstrates value of calibrating to multiple endpoints to determine the correct pMoA. Finally, this study provides a much-needed case study in which the effects of a pesticide on a bird species relevant to ERA were accurately predicted by a DEB-TKTD model.

#### 4.09.B Mechanistic Effect Modelling for Regulatory Environmental Risk Assessment: From Molecules to Landscapes

##### 4.09.B.T-01 Modelling Temperature-Dependent Life-Cycle Toxicity of Thiamethoxam in *Chironomus riparius* Using a DEB-Based TKTD Model

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Thiamethoxam (TMX) is a systemic neonicotinoid insecticide, which is used to protect crops against insect pests. In a recent ecological risk assessment released, the US EPA identified potential concerns primarily from chronic TMX exposure to freshwater aquatic invertebrates. Chironomids were identified as one of the most sensitive taxa. Given the use of TMX in different regions, the toxicity of TMX to the chironomid life cycle at variable environmental temperatures needs to be considered in risk assessment but is not yet well understood.

In this study, we developed a quantitative mechanistic effect model for *Chironomus riparius*, to simulate the species' life history under dynamic temperatures and exposure concentrations of TMX. Laboratory experiments at both constant and dynamic conditions of temperature and TMX exposure were performed for model calibration and validation. The larval dry weight and survival over time, the time to emergence, and the reproduction (as mean egg mass size) were assessed as endpoints. The model design was based on the state-of-the-art mechanistic approach of combining the Dynamic Energy Budget (DEB) theory with toxicokinetic-toxicodynamic (TKTD) modelling. Different assumptions about the size-dependency of *damage* dynamics were explored to adequately reproduce the experimental data. Furthermore, multiple hypotheses about which processes and parameters should be corrected for temperature were tested.

The results showed concentration-dependent effects of TMX on the chironomids' life cycle, including slower growth, later emergence, smaller egg masses, and higher mortality rates with increasing concentrations. Besides an expected acceleration effect on the organisms' growth and development, higher temperatures further increased the adverse effects caused by TMX. With some data-informed modelling decisions (most prominently the inclusion of a size-dependency that makes larger animals more sensitive than smaller ones), the model was parametrised to convincingly reproduce the calibration data. Furthermore, the calibrated model also delivered adequate fits to independent data, which had been collected specifically for validation purposes. While the approach of combining DEB with TKTD modelling has been tested before with other species-toxicant combinations, this study is among the first to rigorously investigate the combination of toxic stress and temperature-dependency with tailor-made extensive experiments.

#### **4.09.B.T-02 Comparing Dynamic Energy Budget Parameters and Traits of Birds for the Extrapolation of PPP Exposure Impacts across Species**

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A major challenge in Environmental Risk Assessment is to evaluate the implications of the results from standard toxicity tests with a limited number of species in the context of species diversity. This is especially an issue with Plant Protection Products (PPP) as potential toxic agents, as a wide variety of organisms is bound to become unintentionally exposed as a result of field applications, while there is little experimental information that can help in estimating the potential impacts of PPP exposure on non-target organisms. In order to bridge this knowledge gap, we use Dynamic Energy Budget (DEB) theory, which is a cohesive, process-based bioenergetic framework describing the rates at which organisms acquire resources from their environments and use the energy and nutrients therein for growth, maintenance, development and reproduction. The dynamic equations of the theory are universal, with taxon-specific modular modifications, and differences among species are essentially differences in parameter values. Although the standard animal DEB model has only 11 core parameters and 4 state variables, these quantities can be used to calculate over 250 traits, implied properties and other derived quantities, such as maximum size, respiration rate and maximum reproduction rate, even if experimental information about those quantities is lacking. In the theory, sub-lethal effects of toxicants are expressed as quantitative changes in energy flows or life history trait values, known as Physiological Modes of Action, which lead to alterations in organismal performance, e.g., reductions in growth and/or reproduction. Accordingly, DEB theory promises a prospect to project known toxic impacts of PPPs on a few dedicated species to other species that are not involved in toxicity testing. The present study focusses on 72 North American birds that may inadvertently be exposed to PPPs. Within the DEB framework, we compare the bioenergetic parameters, traits and derived quantities of those birds with species that are routinely used in reproduction toxicity tests, namely Mallard, Bobwhite Quail and Zebrafinch, and describe the extent to which the latter species are representative of the former, with the ultimate aim to make informed projections of toxic impacts across bird species.

#### **4.09.B.T-03 A Population Model to Assess the Combined Effects of Ionising Radiation and Chemicals on Wildlife**

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A conceptual population model is presented to study the combined effects of ionising radiation and chemical pollutants on wildlife. The model is based on first order, non-linear logistic differential equations combining mortality, morbidity and reproduction phenomena with life history data and ecological interactions. The model includes adaptation as a possible mechanism in order to be able to simulate theoretically potential priming effects at low levels of radiation or chemical concentration. Radiation and chemical-induced damages are represented by a 'repairing pool' mediating between healthy, damaged, adapted and irrecoverable individuals. Damages to population, fecundity and the repairing pool are represented by a linear-quadratic function combining radiation dose and chemical concentration terms. The endpoints of the model are repairable damages (morbidity), impairment of reproductive ability and mortality.

The governing equations of the model are presented and discussed. The model is evaluated with a mixed radiation/arsenate scenario and model simulations illustrate the combined effect of radiation and chemical pollutants upon the sustainability of a hypothetical population, including the influence of adaptation, as well as the capacity of radiation and chemical toxicity to prime for each other, given the assumption that the repair of both radiation and toxicity damages share the same mechanism. A sensitivity analysis of the model illustrates the effects of combining radiation dose and chemical concentration on self-repairing and reproductive ability for the population, exploring cases of antagonism and synergism by varying the relevant model parameters.

This model provides a conceptual framework to assess synergistic effects of mixed radiological and toxic exposures at the population level, suggesting the possibility of a practical regulatory model. The output can be used to inform ongoing debates on the robustness of protection benchmarks in wildlife risk assessment. Future research will enable to draw conclusions about the most restrictive mixed exposure situations in terms of effects to the population, once existing uncertainty in some of the toxicity model parameters has been addressed. It is essential to improve the model with lessons learned from well-established approaches for chemical risk assessment, signalling the direction of future investigations.

#### **4.09.B.T-04 Modelling the Effect of Resource Competition and Feeding Stress on Individual and Population Level Responses in Two Freshwater Shredders**

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Ecological functions are performed by populations of species but toxicity endpoints are derived from single-species toxicity tests. Population responses to individual-level effects can be mediated by other ecological effects such as intraspecific competition for resources. Increasing competition reduces the amount of food available to individuals, affecting their acquisition of energy. For more ecologically relevant ERA, there is a need to extrapolate from individual-level effects to population-level responses to stress, understanding how competition for food affects population-level responses to chemical stress and how this differs between species. *Gammarus* and *Asellus* are freshwater invertebrates that play an important role in the decomposition of leaf litter and nutrient cycling. DEB IBMs for *Gammarus pseudolimnaeus* and *Asellus aquaticus* were used to simulate population-level responses to individual feeding stress (reduced feeding rate) and competition for food. The effect of feeding stress on individual-level responses was compared between *ad libitum* ( $f = 1$ ) and conditions of a reduced resource ( $f = 0.5$ ) at the end of 365 days. Population level responses (change in Biomass production) were simulated over 20 years. Sensitivity to feeding stress increased with decreased resource availability. *Asellus* was more tolerant of decreased availability of food. A 50% reduction in feeding rate resulted in 77% effect on the mean dry weight of individuals for *Gammarus* and a 70% effect for *Asellus* when feeding *ad libitum*.

When the functional response was reduced to 0.5, the effect on Biomass increased to 95% for *Gammarus* but to 81% for *Asellus*. This difference between species was found with Long term population level responses, with a variable resource, where a 50% reduction in feeding resulted in a 35% reduction in the production of biomass for *Asellus* but a 68% reduction for *Gammarus*. Starvation tolerances were the same for both species but simulated *Asellus* had a shorter time to maturity and more reproduction cycles per year, increasing the population tolerance to stress. Individual level effects are not representative of population level responses and their effects on ecosystem functions are affected by food availability caused by competition. The magnitude of effects differed between the two species studied. Life history traits, particularly reproductive cycles affect species' sensitivity to stress at the individual and population level.

#### **4.09.B.T-05 Normal Operating Ranges (NOR) as a Measure to Analyse Arthropod Abundance Variability in Landscape Simulations**

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Arthropod population numbers can change significantly from year to year and season to season due to various factors, like weather or (sub-)annual life cycles. Assessing declining abundances of species therefore proves difficult. Knowing the amplitude of naturally occurring variances in the ecosystem is thus key to assess a potential decline due to newly introduced stressors. Basing such analyses solely on field data would require extensive field studies over a period of years. Additional usage of simulations enables the assessment to be carried out over a longer timeperiod, and additionally to account for e.g. changing weather conditions. We used the individual- based ALMaSS landscape simulation model of Topping et al. (2003), and here implementation of the Bembidion beetle species by Bilde and Topping (2004) to simulate beetle population abundances in a Danish agricultural landscape. ALMaSS simulates landscapes with real past weather data and dynamic crop management while the Bembidion model simulates individual beetles with their livestages and interactions with the landscape and other beetles. To analyse the variability of seasonal abundance numbers, Normal Operating Ranges (NOR) are calculated. The concept of NOR describes the possible range of a measurand (e.g. population numbers) being usually observed in a certain period and (eco-)system. It should thus be described as a tolerance interval covering a defined share of the data with a certain confidence. Abundance data of 130 simulations (30 years each, excluding 10 years of a 'burn-in' period) was overlayed for each day of the year and used for daily abundance NOR calculations for all beetle livestages. Results show clear seasonal changes for each development stage that match expected abundance patterns. Such statistical NOR calculations based on simulation results appear as useful way to analyse the variability of species abundances, if the used model is capable of simulating relevant species traits and phenomena and their variabilities. The use of the NOR concept might be used to analyse stressor impacts, develop in-field verifiable protection goals or the impacts of a changing climate in the future.

#### **4.09.P Mechanistic Effect Modelling for Regulatory Environmental Risk Assessment: From Molecules to Landscapes**

##### **4.09.P-Th311 Physiologically-based Kinetic Models for Bird Species - A Case Study with Fluopyram**

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Fluopyram (FLU) is a fungicide and nematicide used in agriculture to control fungal diseases. In birds, it is mainly metabolized to its benzamide metabolite (FLU-benzamide). The purpose of this study was the model-based evaluation of the toxicokinetic properties of FLU in chicken (*Gallus gallus*), mallard duck (*Anas platyrhynchos*) and in bobwhite quail (*Colinus virginianus*) by simulating and extrapolating egg concentrations in different exposure scenarios. Knowing the egg concentrations in the bird reproduction studies is important for understanding the mechanism that underlies the effects of FLU in these studies. A physiologically-based kinetic (PBK) model of FLU was built using the generic bird PBK models developed with the Open-Systems-Pharmacology Suite (OSP Suite with PK-Sim® and MoBi®). The PBK model includes the physiology (e.g., blood flow rates, organ weights) of both the chicken and bobwhite quail and FLU-specific parameters were fitted to residual data in chickens. To characterize metabolism of FLU, *in vitro* measurements of chicken and quail metabolism have been included into the model. To investigate egg concentrations in a non-constant exposure scenario, a pulse of exposure was simulated, opposed to the controlled quail reproduction studies using constant exposure over multiple months, which is often unrealistic when estimating effects in the environment. The pulsed exposure was based on a DT50 that was derived from residue data on insects for FLU. The model was able to predict FLU and its FLU-benzamide metabolite in tissues and eggs. The overall performance of the chicken model was very good and 96% of the simulated data points were within the 3-fold range of the observed data. Overall, toxicokinetic properties were comparable between chicken and quail, despite differences in *in vitro* metabolism. This is explained by blood-flow limitation of liver metabolism. Uncertainties related to data gaps on species-specific properties such as the unbound fraction of compound in plasma, extrahepatic metabolism and active renal excretion remain. The PBK model predictions are relevant for assessing concentrations that could lead to possible adverse effects on the bird's reproductive performance and offspring. Due to the separation of species- and compound-specific parameters, PBK models are generally well-suited for species extrapolation, as presented in this study.

##### **4.09.P-Th312 Application of Quantitative Structure-Activity Relationships (QSAR) For Physiological-Based Toxicokinetic (PBTK) Feed-To-Fillet Transfer Modelling of Contaminants in Feed to Atlantic Salmon**

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The European Union has set maximum residue levels for undesirables in feed ingredients and food from animal origin, in order to protect animal health and consumer safety. Physiological-Based Toxicokinetic (PBTK) transfer modelling of contaminants from feed to animal food products is an essential component for harmonization of legislation of undesirables throughout the food production chain. Several PBTK models for the prediction of transfer of specific compounds or compounds groups in Atlantic salmon fillet exist already. However, these are largely based on *in-vivo* experimental data. Following these models, the current project aims to develop a generic *in-silico* feed-to-fillet transfer model for feed contaminants by merging *in-silico* methods with the existing PBTK salmon models.

To achieve this, quantitative structure-activity relationships (QSAR) are used for the model parametrization by relating molecular descriptors to the key model parameters. In addition, selected experimental parameters are considered as covariates. One of the key model parameters is the uptake rate. A literature search was conducted to obtain a database of gut absorption efficiencies that is used as a basis to train the model. Different modelling approaches, such as partial least squares (PLS) or machine learning algorithms are used and compared regarding their predictive power.

Preliminary results show that the chemical structure alone can not account for the uptake rate, but experimental parameters need to be taken into account. In addition, main factors of uncertainty that were identified are the limited data availability and as well as biological variability.

The generation of a generic feed-to-fillet model would allow for quick assessment of potential accumulation of feed contaminants in salmon fillet and greatly facilitate assessment of food safety.

#### **4.09.P-Th313 Toxicokinetic and Toxicodynamic Modelling for Non-Monotonic Fluoxetine Toxicity in *Caenorhabditis elegans***

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With increasing antidepressant prescriptions worldwide, a growing number of organisms is exposed to these pharmaceuticals in the environment. Antidepressants are neuroactive compounds and can therefore induce sensitive behavioural effects that could also propagate effects at a population level. Dose-response relationships of antidepressants sometimes show non-monotonic patterns. Theories explaining non-monotonicity link to both toxicokinetic and toxicodynamic processes. Therefore, our study aims at developing a toxicokinetic/toxicodynamic (TK/TD) model for the toxicity of the antidepressant fluoxetine in *Caenorhabditis elegans* to gain a better mechanistic understanding of the dose-response relationship over time. Behavioural experiments with a wide range of fluoxetine concentrations were conducted to study the response for three different endpoints: general activity, feeding and chemotactic behaviour. Mono-, bi- and tri-phasic non-linear regression models were applied to test for non-monotonicity. Following this, 24 hour uptake, metabolism and elimination experiments were conducted for fluoxetine and its active metabolite norfluoxetine. A TK/TD model was developed with three compartments: the medium, *C. elegans* and its food source *E. coli*. Results of the behavioural experiments show non-monotonic dose-response relationships for both chemotaxis and activity. Activity was tracked over a 24 hour time period and we observed stabilization of the behavioural effect at 100 mg/l, a recovery at 10 mg/l, but a gradual continued increased effect at 10 ng/l. Results of the uptake, elimination and metabolism experiments demonstrated a rapid uptake of fluoxetine that was further increased when *E. coli* were present. The internal concentration of fluoxetine decreased after 5h (clean medium) or 10h (with *E. coli* present) under constant exposure, likely because of an increasing detoxification rate over time. This could be a reason for the recovery or stabilization of the effect at later time points. These first results indicate possible explanations for non-monotonic responses to fluoxetine, but they can be better interpreted with a TK/TD model. During the conference, we aim to present the results of the TK/TD model. Future experiments will also focus on repeated exposure, to see how both the TK and TD aspects of the model will develop in a more environmentally relevant exposure scenario.

#### **4.09.P-Th314 Evaluating TK Compartmental Models Performance as Predictors of Internal Concentration in Environmentally Relevant Species**

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The large number and variety of chemicals in use today presents a major challenge in determining environmental safety for chemicals while minimizing the generation of new animal toxicity data. One proposed strategy for advancing the pace of environmental risk assessment is to use new methods which allow for higher throughput, including *in silico* and *in vitro*, which are generally referred to as New Approach Methodologies (NAMs). However, NAMs require robust toxicokinetic models, which allow the conversion between internal (at target site) and external (water) levels of chemicals in environmentally relevant species. *Daphnia magna* is a key model organism for ecotoxicology. However, quantitative toxicokinetic data for invertebrates, including *D. magna* are limited, resulting in a lack of robust Physiologically based kinetic / quantitative In Vitro to In Vivo Extrapolation models for invertebrates. Current models are chemical specific – derived by fitting individual chemical data, with restricted application to other chemicals.

This work addresses the current quantitative approach limitations. *D. magna* toxicokinetic study data were collected from 14 studies across 26 chemicals. The *D. magna* study data were standardized and combined with a *P. promelas* and *O. mykiss* specific dataset, containing a further 23 studies across 39 different chemicals.

A cross-species compartment-model based evaluation framework was developed using already well-established modelling concepts. Given the proposed cross-species evaluation framework, we show that concentrations are better predicted in *D. magna*

than in *fish*. This could suggest the simple model is unable to capture the uptake dynamics of a more complicated system like the fish whilst being able to capture the simple diffusion of the chemical into the *D. magna*. Further analysis of the *D. magna* prediction errors suggests that species performance differences are likely driven by the reliance on the octanol-water partition coefficient of the chemical, independent of time-course assumptions and physiological differences between the organisms. Our study shows the ability to generate robust predictions to support environmental safety decisions, not only for *D. magna* but any organism with minimum data requirements, with significant implications for next generation environmental risk assessments.

#### 4.09.P-Th315 A Framework For Algae Modelling in Regulatory Risk Assessment

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The use of Toxicokinetic-Toxicodynamic (TKTD) modelling in European regulatory risk assessment of plant protection products is rapidly gaining momentum, especially following the endorsement of the European Food Safety Authority (EFSA) in the 2018 opinion on TKTD modelling for use in risk assessment. This document outlines the regulatory context, the general model requirements, and the readiness of a series of established individual models for use in aquatic risk assessment [1]. Despite the fact it does not have a toxicokinetic (TK) component, the Weber et al. model of 2012 for green micro-algae is one of three TKTD models for primary producers that is discussed in the report. The model, also referred to as the SAM-X model, was designed for use in risk assessment of plant protection products (PPPs) to account for the effects of time-variable concentrations. However, the step-by-step workflow to link exposure profiles to risk assessment decisions via algae TKTD modelling has not yet been made clear. We here revisit the SAM-X model to present a modelling framework developed in accordance with the EFSA guidelines for regulatory submissions. We here demonstrate the power of TKTD modelling for use in Tier 2C risk assessment for algae. Based on the generation of a calibration and validation dataset, we were able to simulate over 11 thousand standard OECD TG 201 algae growth inhibition tests. Adhering strictly to the format of the standard test we changed only the concentrations – replacing constant exposures for time-variable exposures defined by FOCUS model outputs. Our implementation of the moving time window neatly avoids any of the issues caused by longer exposure times in algal cultures, such as over-crowding and nutrient stress. This permits us to assess the effects of long exposure profiles without introducing the unwanted complexity of multiple stressors at lower tiers of the risk assessment. We found that the moving time window approach is consistent with risk assessment under tier 1 for cases where the RAC is exceeded for three consecutive days. However, for peaks where RAC is exceeded for shorter durations, the approach offers a suitable refinement option for tier 2C. Our implementation of this modelling framework for the case study chemicals chlorotoluron and isoproturon demonstrated examples of both low risk and high risk for example FOCUS profiles.

#### 4.09.P-Th316 Calibration and Validation of Algae TKTD Modelling for Risk Assessment

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Advances in ToxicokineticToxicodynamic (TKTD) modelling have made refinement with models an increasingly recognized option for risk assessors. One of the few TKTD models available for plant species is the SAM-X algae model that, when properly calibrated and validated, can predict algae responses to time variable concentrations of a target compound. The data generation for calibration and validation of this model, however, is not straight-forward. The European Food Safety Authority's (EFSA) opinion on TKTD modelling for use in risk assessment states that "...the largest drawback for implementing [the algae model] in pesticide risk assessment is that the flow through setup used in the existing example and needed to simulate long term variable exposures of pesticides to fast growing populations of algae has not yet been standardised, nor has the robustness of the setup been ring tested." The overall conclusion in the report is that the model therefore, is not yet ready for use in risk assessment. The present work is a direct response to the comments made by EFSA, and we here aim to demonstrate an explicit example of how time-variable data generated from seven laboratories can be used to calibrate and validate the SAM-X model. Further, we aim to demonstrate how we, following the guidance from EFSA, can use modelling to simulate OECD style experiments to extrapolate to longer exposure profiles using the moving time window framework. This modelling exercise is an answer to the comments to the algae model in the EFSA report on TKTD modelling. We provide a thorough evaluation of the data generation methodology, and we scrutinize the robustness of the data throughout the modelling framework – from the early stages of model calibration to the later steps of validation and extrapolation to longer and more realistic exposure profiles.

#### 4.09.P-Th317 An R package for Calibration, Validation, and Effect Endpoint Calculation of Toxicokinetic-Toxicodynamic Models

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Toxicokinetic-Toxicodynamic (TKTD) modelling is increasingly used in European regulatory risk assessments of plant protection products, after publication of EFSA's scientific opinion on TKTD modeling. This creates a need for software that allow for quickly and smoothly going through the whole workflow required for regulatory submissions: from model calibration and validation, over effect calculation for exposure scenarios, up to calculation of the respective regulatory endpoints such as LP50 and EP50 values.

We developed an R package that implements the whole workflow for *Lemna*, GUTS and DEB models. Our goal is to ease the handling of TKTD models for scientific as well as for regulatory use cases. Importantly, the workflow implementation is independent of a specific model, thus all TKTD models are handled uniformly. In addition, the intuitive workflows can be extended to use custom models implemented by users of the package.

The model equations are implemented as C code which makes the package computationally efficient and decreases computation time by more than a factor of 1000 compared to pure R code.

We intend to publish the package as Free and Open Source Software to further foster the prosperity of TKTD modeling.

#### 4.09.P-Th318 How to Include Temperature in Mechanistic Effect Models

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Temperature is a natural variable, never constant under field conditions. And as global climate change continues, extreme temperature events are expected to increase in frequency. Therefore, prospective environmental risk assessment needs to consider the potential influence of those changing temperature conditions on pesticides' toxicity. For this, the variation of temperature should be considered in mechanistic or process based models like toxicokinetic-toxicodynamic (TKTD) models for chemicals. There are two different ways to include the influence of temperature on chemical effects. Firstly, temperature can be interpreted as a modulating factor, influencing the TKTD processes of the chemical. Secondly, temperature can be approached as an individual stressor, in addition to the chemical stress. Even though in reality, it might be a combination of both, looking at these two different approaches separately will help to understand the mechanisms of the combined stressors, chemicals and temperature. Temperature has been successfully included in TKTD models as a modulating factor via the Arrhenius equation, however, with limiting applicability regarding temperature conditions outside of the thermal tolerance of the organism. To explore how to include those extreme (i.e., stressful) temperatures, we developed a module that allows to include temperature as a separate stressor in TKTD models for chemicals. We translated the damage model as used for chemical effects in the General Unified Threshold model of Survival (GUTS), to a damage model for the effects of temperature. We will present the assumptions and mathematical approach of this model and test its performance to predict experimental data. Furthermore, we implemented the new temperature damage model in the mixture GUTS framework to assess the survival of combined exposure to a temperature and a chemical stressor.

#### 4.09.P-Th319 Has GUTS-RED Still Too Many Parameters? Comparing Data Explainability and Parameter Robustness of GUTS Variations for Arthropods

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The General Unified Threshold Model of Survival (GUTS) of Jager et al. (2011) managed to unify many existing toxicokinetic-toxicodynamic (TKTD) models into a consistent framework to analyse stressor impacts on individual survival based on laboratory test data. Using the full GUTS model requires internal concentrations, making its usage unfeasible in many cases. Two reduced GUTS variations (GUTS-RED-SD & -IT) emerged as models of choice to analyse standard ecotoxicological data sets, covering only external stressor concentration and animal survival. These reduced models use four parameters to “learn” the link from external stressor to death from data by using an internal “damage” as substitute for actual stressor concentrations. Data explainability is good in many use cases, making GUTS ready to be used for regulatory risk assessment according to EFSA (2018). However, resulting parameter values tend to correlate strongly with each other and are thus neither interpretable in their absolute values nor robust. Robustness could be defined as when parameter values emerge from similar datasets with identical or very similar values. When refitting GUTS to slightly altered input data, the fits look comparable, while often using a completely different set of (absolute) parameter values. With a robust set of parameters the values would not change significantly when the datasets are near comparable.

Performance in terms of model accuracy and parameters robustness of the two GUTS-RED models is therefore compared to other variations of GUTS. Potential variations include for example using the external concentration directly as dose metric for the death mechanism. Other variations are generated by fixing individual parameters in the model. Parameter robustness is quantified by refitting the model to resampled datasets. All considered GUTS variations are compared based on data for different pesticide stressors and arthropod species to identify the most suitable GUTS variations concerning data explanation and robustness. First results suggest that variations with three parameters reach a comparable model accuracy, while showing a higher robustness and better model selection parameters like the Akaike information criterion.

#### 4.09.P-Th320 DEB Modelling of Anuran Amphibians for Setting a Normal Operating Range for Metamorphosis Duration

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Amphibian tests, required by EFSA (OECD TG 231, 241, 248) are aimed at characterizing the thyroid axis, which is known to affect metamorphosis in amphibians. However, metamorphosis in amphibians is well known to be a plastic process and amphibians change rate of metamorphosis in response to various environmental stressors. This means the speed of metamorphosis from tadpole to froglet is a trade-off between fast development and size of the froglet. Specifically for anuran species that depend on fish-free, and hence often non-permanent water bodies, metamorphosis is necessary to be completed before the water body dries up. In such cases the tadpole trades the risk of death due to lacking the necessary adaptations to live on land when the pond is dried up against a small size at metamorphosis and co-occurring selective drawbacks (e.g. higher risk of desiccation or being predated upon).

EFSA has used models to assess the normal operating range (NOR) of natural dynamics, e.g. for honeybees. Here, we use an adapted DEB model to assess the normal operating range of plastic processes under different environmental conditions.

Mechanistic modelling is gaining importance in the field of ecology and ecotoxicology. Models based on the dynamic energy budget (DEB) theory are becoming the standard for the assessment of stressors on sublethal endpoints (e.g., size, mass, etc.).



However, the currently available DEB models for amphibians are not suitable for this purpose as they lack a specific treatment of the metamorphosis process and adaption. Consequently, they cannot simulate the plasticity regarding various stressors. Here we propose a robust and general model that can describe amphibian metamorphosis within the DEB framework by incorporating modules inspired by the DEB modelling of holometabolic insects (that also undergo metamorphosis). This implementation allows the qualitative and quantitative description of the size changes of the tadpoles under normal conditions and the effects that stressors may have in modifying development. We show examples of model calibrations for anuran species native to Europe and for the African clawed frog, *Xenopus laevis*, a species widely used in laboratory testing.

#### 4.09.P-Th321 Building Dose-Response Relationships from Standard Rat Toxicity Studies With DEB-TKTD Models

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Most studies used for setting endpoints for wildlife risk assessment of small mammals are performed primarily for human toxicology, where the aim is to set no observed effect levels (NOEL). Approaches based on dose response curves (DRC) are currently the standard for analysing such studies e.g., the recently updated benchmark dose (BMD) approach guidance by EFSA. However, in order to determine an accurate NOEL, the design of these studies is skewed towards more replicates at low doses rather than including more, higher doses, which would benefit the application of DRC. Additionally, DRC approaches have inherent limitations: they are applied to only one endpoint, only for the most relevant time point and extrapolation is based purely on a statistical relationship, disregarding any links with underlying mechanistic hypotheses or theories.

Dynamic Energy Budget (DEB) models, coupled with Toxicokinetic-Toxicodynamic models (DEB-TKTD), are recognized as an novel tool for risk assessment. DEB-TKTD models approach toxicity from a physiological/metabolic perspective, which allows better quantitative predictions due to a mechanistic understanding of toxicity. The DEB-TKTD framework allows simultaneous calibration on multiple endpoints over time (typically survival, growth and reproduction), which increases the biological relevance of the toxicity predictions and could lead to more robust effect levels based on data for low dose levels in the toxicity studies. The aim of this study is to explore whether DEB-TKTD modelling can be used to establish dose-response relationships for use in risk assessments and highlight their advantages over DRC approaches.

Existing (sub)chronic toxicity studies (i.e., 90-day and 2-generation studies) on the rat (*Rattus norvegicus*) were used to parameterize a DEB-TKTD model. Control treatments were used to calibrate the physiological (DEB) part of the model. After accounting for the effect of toxicant-induced reduction of feeding, the most appropriate physiological mode of action (PMoA) was determined. Growth and reproduction data in the treatments were used to derive the most appropriate PMoA. Finally, effect levels for the studies were derived using both the DEB-TKTD model and the BMD approach and the accuracy and uncertainty of both approaches were compared. The outcome of this comparison is used to discuss the advantages and challenges of both approaches as a tool for environmental risk assessment.

#### 4.09.P-Th322 Analysis of Sublethal Effects of Pyraclostrobin in Mysid Shrimp (*Americamysis Bahia*) Using a DEB-Based TKTD Model

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Pyraclostrobin is a widely used fungicide from the class of strobilurins. In this study, we refine the chronic risk assessment for aquatic invertebrates, by using a quantitative effect model for pyraclostrobin and mysid shrimp (*Americamysis bahia*). At its core, the effect model represents a DEB model which is complemented by a TKTD module, accounting for lethal and sublethal effects. Experimental data from one acute and two chronic studies were used to calibrate and validate the model. Besides effects on survival, both chronic studies showed effects on growth and reproductive output of the mysids. For the unstressed DEB model, a model entry for *A. bahia* in the *Add-my-Pet* online repository served well, since it matched the experimental control data with high accuracy. Survival effects were modelled using a stochastic death model, whereas sublethal effects were captured best using a linear effect-above-threshold assumption for both the energetic costs for somatic maintenance and the costs for creating new eggs. While the sublethal effect data used for the model validation were adequately reproduced by the model with a slight tendency to effect overestimation, effects on survival were clearly overestimated by the model. This discrepancy, however, follows directly from a mismatch between the calibration and validation data originating from different studies. Nevertheless, considering the consistent overprediction of adverse effects, the model is conservative.

In a final step, we used the model to simulate the life cycle of mysids with realistic exposure profiles using a moving-time-window (MTW) approach. This method allows identification of the most adverse exposure window per profile, resulting from a critical combination of endpoint-dependent life-stage sensitivity and timing and magnitude of exposure. We then determined exposure multiplication factors that would result in a 10% effect on the most sensitive endpoint (here: reproduction) in the most critical window of each profile (EP<sub>10</sub>). All EP<sub>10</sub>-values for the relevant scenarios were above 10, indicating low risk for the proposed application pattern, including some additional risk mitigation measures such as drift reducing nozzles and/or buffer zones. In summary, we were able to demonstrate that a combination of laboratory experiments and mechanistic effect modelling can be used to aid environmental risk assessment in a meaningful way.

#### 4.09.P-Th323 What Mechanistic Effect Models Can and Cannot Tell Us About the Variability in the Real World

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Mechanistic effect models (MEMs) are increasingly used to support ecological risk assessments of plant protection products (PPP). The European Food Safety Authority (EFSA) has been supporting the developments of these important tools by providing several scientific opinions as well as public reviews of specific mechanistic effect models. Most recently, EFSA issued an opinion on background variability, based on the honey bee colony model BEEHAVE, an important aspect providing context for determining acceptable effects from exposure to PPPs. Models are in many cases calibrated and validated to represent average system behaviour and are thus not designed to make predictions on the variability of system behaviours. The exciting possibility to apply MEMs to determine normal operating ranges requires a thorough understanding of the variability implemented and observed in MEMs, namely its causes, its ranges, and how it compares to empirical observations as well as a definition of relevant spatial and temporal scales. Finally, a model's defined domain of applicability needs to be considered. It is currently still unclear how much variability can and should be represented in mechanistic effect models relative to what is observed in empirical systems as well as how well models can explain observed variability. To this end, we investigated models used within the PPP risk assessment arena, that accounted for sources of variability and that were validated with empirical data from lab and field experiments. We found that variability in control conditions was caused more often by parameters based on observed data and rarely by generic stochastic implementations. Model output generally showed less variability than the empirical data used for validation and the difference was especially visible when field data were used. Models were also successfully applied to explore the causes of variation in empirical data. As recommended by EFSA in their Scientific Opinion on good modelling practice, it is important to assess whether a given problem lies within a model's domain of applicability. Furthermore, there are other methods available which have been used for uncertainty analysis and probabilistic risk assessment (e.g., WEBFRAM, EUFRAM). In summary, when setting normal operating range of an empirical system then empirical data, MEMs, and non-mechanistic probabilistic methods should all be evaluated.

#### **4.09.P-Th324 Endocrine Disruption: How to Perform a Hazard Assessment with Population Models?**

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Regulation 1107/2009 states that agrochemicals identified as Endocrine Disruptors (EDs) are banned for use in the EU. The ED identification criteria defined in Commission Regulation (EU) 2018/605 rely upon a hazard assessment. For non-target organisms, to be identified as an ED a substance must result in an adverse effect on a population as the result of an endocrine-mediated mechanism. Critically, adverse effects are commonly reported on individuals (e.g. fecundity in Fish Short Term Reproduction Assay) and so a method is required to extrapolate from the organism level to that of the population. Population modelling is one such method, however, ECHA-EFSA (2018) state that "...generally these models are more suitable for risk assessment purpose..." but provide no guidance on how such a method should be used in the context of EDCs.

We present the results of two modelling approaches that are consistent with the concept of a hazard assessment. In one, we do not consider exposure at all, effectively performing a sensitivity analysis on ED-relevant endpoints at a range of effect magnitudes over a period of 10 years. For this, we used three fish population models, the endpoints sex ratio, fecundity, fertilisation rate, growth and behaviour, with a range of effect magnitudes of 10, 20, 50 and 90%. We performed modelling for each endpoint individually and in combination. In the second, exposure is taken into account using regulatory fate models to predict concentrations in the environment following the proposed use of a product. In this instance, we identify an effect-threshold, above which effects are imposed and below which no effects are imposed. This threshold was based on the lowest NOEC from all available ED-relevant studies with the substance. This is still a hazard assessment, but the threshold is not 0 as in the first approach. This makes more use of the available data on a molecule and its proposed use and is justified for regulatory use as the Regulation 1107/2009 states that "*negligible exposure*" is acceptable.

We propose that these two modelling approaches could be used in conjunction with one another: the first method based on simple look-up tables as a screening assessment and the second method as a higher tier refinement making full use of the substance-specific data package.

#### **4.09.P-Th325 Simulating Life-Cycle Toxicity in Chironomus Riparius With Realistic Dynamic Exposure Profiles and Variable Temperatures Using a Moving-Time-Window Approach**

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Insecticides are applied to treat many crops all over the world. Freshwater aquatic invertebrates are often identified at risk regarding exposure to insecticides from agricultural runoffs, with chironomids identified as one of the most sensitive taxa. To better quantify the chronic toxicity of a neonicotinoid insecticide in chironomids, we calibrated an individual-level mechanistic effect model for *Chironomus riparius*, which accounts for both time-varying temperature and insecticide concentration.

We used this recently developed model to simulate *C. riparius*'s life cycle with realistic exposure profiles and variable temperatures using a moving-time-window (MTW) approach. In this approach, a species' life cycle is repeatedly simulated, each time with a slightly shifted starting point in the exposure profile. This method is particularly relevant when the life cycle of a test organisms is shorter than existing exposure profiles used in risk assessment. It allows to determine the most adverse exposure window, resulting from a critical combination of (endpoint-dependent) life-stage sensitivity and (timing and magnitude of) chemical exposure. Besides the individuals' survival, their growth, time until emergence, and potential reproduction were assessed as endpoints. While the duration of each life-cycle simulation is typically set to a fixed length (mimicking a real-life experiment), in this study, the durations were corrected to account for temperature-dependencies in physiological rates, i.e., the time window corresponded to a physiological state, rather than the length of a regulatory experimental study. This resulted in

shorter windows at higher temperatures. Furthermore, while MTW simulations have so far been used in automated routines to seek out exposure multiplication factors (EMFs) resulting in a pre-defined effect strength, analogous to summary statistics like EC<sub>x</sub>, recent research has raised concerns to this approach in cases where non-monotonous relationships exist between EMFs and the endpoint of interest. Therefore, in this study we created comprehensive EMF–effect profiles for each endpoint to determine critical EMFs in a more robust way.

With this study, we have contributed to the further development of the MTW approach, which is a highly valuable approach for ecological risk assessment of agricultural chemicals.

#### **4.09.P-Th326 Environmental Risk Assessment at Landscape Level – Open Questions**

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Environmental risk of pesticides depends on the landscape under consideration. Through ecological modelling at landscape-scale we can devise holistic strategies for how to manage agricultural landscapes to optimize across ecosystem services whilst considering all relevant factors. What is unclear is how protection goals can be operationalized for decision making in a landscape level risk assessment.

Landscape level risk assessments require definition of the baseline scenarios against which the impact of an added stress factor like pesticides can be measured, including: spatial and temporal scale, landscape structure and composition, land use, natural factors (soil, weather, flora and fauna, habitat structures and sizes), agronomic practices, land management, risk mitigation and crops. Other stressors, natural and man-made, as well as their intensities and durations, need to be clarified. To some degree these baseline scenarios could be generalized, which means we would not need infinite numbers of scenarios for all combinations of species, crops and landscapes.

Ecological consistency needs to be maintained in the definition of scenarios, for example as to the type of plant communities potentially in the vicinity of fields where a certain crop could be grown or which data sets for soil, water bodies, land-use and climate are appropriate. Combined spatially distributed exposure and ecological modelling could track the exposure experienced by organisms and inform effect models predicting population dynamics. Baseline scenarios could also represent organic farming practices to compare with conventional farming or integrated pest management approaches. Endpoints for environmental risk assessment at landscape scale would differ from lower tiers but it is not clear which ones are meaningful and how they should be assessed.

There is an opportunity to use landscape level environmental risk assessment to also explore different agronomic management options, including non-pesticide related options (e.g. tillage vs. no till), the effectiveness of different mitigation options, alternative agricultural practices and landscape management options. Most importantly, landscape level risk assessment enables a more realistic and ecologically relevant risk assessment, for example by considering effects of other stressors (e.g. diseases, agronomic practices, land management). It may be possible to introduce landscape level risk assessments in a stepwise manner.

#### **4.09.P-Th327 Assessing Ecological Risk from Mercury Exposure Across the South Florida Landscape from the freshwater Everglades to Coastal Marine Waters**

**Darren G Rumbold, Florida Gulf Coast University, USA**

Mercury (Hg) was first recognized as a problem in Florida's freshwater Everglades in the late 1980s. The first fish consumption advisory was issued in 1989. It did not take long, however, to recognize that the problem extended beyond the freshwater marshes out through the estuaries and into south Florida's coastal waters. The first advisory for marine waters, issued in 1991, urged individuals to limit their consumption of sharks. Over the next 30 years, research on Hg biogeochemistry focused mainly on the freshwater Everglades. Several ecological risk assessments (ERAs) done during this period focused exclusively on the freshwater system, primarily because of the concern that efforts to restore the Everglades could worsen the problem. Hg levels have declined in certain areas of the Everglades but hotspots remain with tissue residues in 2021 still exceeding 3 ppm in some fish and 7 ppm in some reptiles. Much of the system remains under limited or no fish consumption advisories. Moreover, declines have not been observed in the marine environment with over 60 species of recreational or commercially important fish species currently under advisories. Hg concentrations as high as 4.5 ppm have been reported in sharks and as high as 42 ppm in feathers from nestlings and 96.5 ppm from adult ospreys (*Pandion haliaetus*). This presentation synthesizes data from both state-run monitoring programs and academic studies to characterize the risk to various taxa (e.g., fish, birds, reptiles, mammals) across south Florida's landscape using three lines of evidence (LOE): 1: based on fish-tissue Hg concentration as the media of exposure; 2: based on tissue residues within the ecological receptors and, 3: based on eco-epidemiological wildlife surveys. Together these LOEs suggest that biota in certain regions of south Florida continue to be exposed to levels of Hg that pose a threat. While eco-epidemiological wildlife surveys of Hg impact on health were the most direct method to assess risks, they were rare. Government agencies frequently monitor Hg in various media (often in support of consumption advisories for human health protection); however, they traditionally do not explicitly look for effects. Unfortunately, without improved estimates of the frequency of these sublethal responses and the probability of ecological mortality, risks go underappreciated by resource managers who may view any uncertainty as justification for taking no action.

#### **4.09.P-Th328 Food and Density Dependence in *Chironomus riparius* (Diptera: Chironomidae): Laboratory Experiments To Inform Population Modelling**

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For ecological risk assessment of anthropogenic stressors, individual-based population models are an effective tool to extrapolate from effects observed on individuals in laboratory experiments to populations exposed under field conditions, integrating different environmental influences on the physiology and population dynamics of a species.

In addition to many dynamic environmental parameters, the capacity of populations in the field is often determined by density-dependent processes, which must be implemented and parameterised for a realistic simulation of population dynamics. However, mechanisms driving density dependence in populations are not always known. Here we present results on the study with the dipteran species *Chironomus riparius*, for which density dependence experiments were conducted in the laboratory. Data on the impact of different food concentrations and larval densities on developmental rate, hatching success, reproductive performance, and larval mortality were collected. Experiments with different food quantities per individual were used to reflect the effects of fluctuating food supply in the environment as well as exploitative competition between individuals, while direct density-dependent interactions were analysed with experiments where food availability per individual was held constant, but systems contained different densities of individuals.

The experimental results were used to parameterise an individual-based population model for *Chironomus riparius*, whose physiological dynamic energy budget (DEB) module had previously been calibrated with laboratory data. This population model, which is also a sub-module of the ecosystem model STREAMcom, was then validated using field data at the population level. The lack of robust data on population regulation and relevant loss rates of larval and adult stages in the environment are often the biggest data gaps in population modelling of non-target organisms in the field. Reducing the uncertainty in the processes of density-dependent development and mortality rates will facilitate calibration of the remaining data gaps and help increase confidence in applying such population models to more realistic environments, such as aquatic outdoor mesocosm studies, for ecological risk assessment.

#### **4.09.P-Th329 DEB-IBM-Based Population Modelling of the Non-biting Midge *Chironomus riparius* (Diptera: Chironomidae)**

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Population models are powerful tools for predicting species population dynamics for different environmental scenarios under consideration of natural and anthropogenic stressors, and have been increasingly used in ecological risk assessment, often coupled with TKTD effect models.

Here we present a model concept for simulating populations of a species of non-biting midges, *Chironomus riparius*, with the aim of extrapolating ecotoxicological laboratory data to field scenarios. This concept combines individual-based models (IBMs), based on physiological dynamic energy budget (DEB) modules and complemented by toxicokinetic-toxicodynamic (TKTD) effect models for lethal and sublethal processes, driven by dynamic environmental conditions. The core of the model is an individual DEB-TKTD model that has been calibrated for individual life cycles using laboratory tests at different temperatures. This population model is also a sub-module of the ecosystem model STREAMcom, which simulates benthic macroinvertebrate communities in lentic and lotic water bodies and is currently used to model communities in aquatic mesocosms.

In developing such complex models, simplified assumptions have to be made about the relevant processes in population dynamics to be represented in a model concept. In addition to external factors such as weather conditions and food availability, population dynamics relevant processes such as feeding behaviour, larval background mortality, and adult loss rates in the field due to dispersal or predation need to be considered. While physiological modules or effect models such as the TKTD model GUTS are at least conceptually easy to parameterise with laboratory data, it is much more challenging to quantify relevant processes at the population or ecosystem levels, such as density-dependent growth and reproduction, or food availability, which ultimately determine the dynamic capacity of the population throughout the year.

We present a modular model framework for a *Chironomus riparius* population model and discuss strategies for model structure development and parameterisation, also in the context of available field data for calibration and validation. We also present model analysis and discuss how those can be used to inform model development.

#### **4.10 New Approaches, Methodologies and Policies in Environmental and Human Health Risk Assessment**

##### **4.10.P-Tu354 Characterization of Microplastics in Beach Sediments Points to the Need for a Precautionary Approach to Plastic Use**

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Data on the harmful effects of microplastics (MP) are not fully available and there is a need to control the microplastic pollution. Characterization of microplastics helps in understanding the impacts of these emerging pollutants on the environment and helps in executing the most effective reduction measures. It is important to formulate and adopt suitable environmental forensic techniques to track the pollution. Environmental forensics helps in identifying the pathways and sources of pollution which is required for implementing the control strategies. Existing frameworks on sampling and analysis of plastics and MP in marine environment were studied in detail. Field investigations were carried out on the coast of Kerala, India, following the existing guidelines with appropriate improvisations to respond to the specific requirements of forensic application. From the analyses results and the experience gained from the site sampling, forensic site investigation protocol was devised for MP contaminated site. The forensic investigation strategy developed was capable of tracking down the sources of a significant portion of MP sampled from the strandline. From the forensic analysis carried out on the MP sampled from Kerala coast, various sources of MP that are either least useful or can be easily eliminated were identified. A microplastic pollution control strategy targeting the easily addressable

sources is expected to be more efficient, both in terms of resources spent and public acceptance. The protocol developed in the study helps to regulate the MP pollution by applying precautionary principle.

#### **4.10.P-Tu355 Importance Of Precautionary Approach In Massive Firework Display Releases- A Case Study From Kerala, India**

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Fireworks displays (FWD) which are an integral part of many festivals, releases highly hazardous and toxic chemicals to the atmosphere resulting in air pollution leading to severe risks to human health and the ecosystem. The final deposition of these elements on soil and subsequent migration to the subsurface and to groundwater aggravates the issue. It is important to conduct forensic studies to fix the share of FWD to the air quality deterioration during festival seasons and to analyse the illegal use of banned chemicals in the fireworks. A precautionary approach is important in the forensic analysis of such environmental crimes. The air quality due to various activities of the festival, including the massive fireworks display during 2019 episode of *Thrissur Pooram*, a world-renowned festival of Kerala, India, where millions of people gathering to witness the festivities, is taken as a case study to analyse the impacts of FWD releases and other festival related activities. In addition to the monitoring of the parameters PM, SO<sub>2</sub> and NO<sub>x</sub>, chemical speciation of the air samples was carried out to check the presence of banned chemicals in these samples. High concentrations of PM<sub>10</sub>, SO<sub>2</sub>, NO<sub>x</sub> and metals were observed in the air on these days compared to the normal values for the city on non-festival days. Analysis of air samples collected after 18 hours of FWD showed a declining trend for the pollutants. The elements that have shown significant increase in concentration after the FWD- Al, B, Ba, Ca, Fe, K, Li, Mg, and Zn- are the ones used in fireworks as colourants, fuel, oxidiser, chlorine donor, etc. Since the vehicular traffic and all other commercial activities were stopped in the nearby area during the festival days, and there is no industry in the locality that could have contributed these metals, it is reasonable to report that it is contributed by the FWD and the soil particles. Sb, Li, Hg, As, Pb, Ba and strontium chromate found in the analysis were chemicals banned for use in fireworks by the Honourable Supreme Court of India. Hence these metals can be considered as the indicators of blatant violations of the orders issued by the Court and of the precautionary principle. Safety of the people is very important as hundreds of thousands of people assemble to witness the festivities. The study points to the need for research to generate new and economic formulations for fireworks that are green and safe, strictly adhering to the precautionary principle.

#### **4.10.P-Tu356 A Forensic Investigation of a Contaminated Land at Kerala, India**

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Contamination of the natural environment has been viewed more seriously ever since its impact on human health became clear and quantifiable. This has led to multitudes of legislations getting enacted in different countries for conserving and protecting the natural environment. An underlying principle of most of these legislations is 'polluter pays'. However, to make the polluter pay for the damages caused to the environment, the responsibility needs to be fixed to the satisfaction of the country's legal system. Environmental forensics provides with a systematic scientific approach for fixing liability in a pollution episode. In this study, the forensic investigation of a contaminated land, Kuzhikandam, Ernakulam district of Kerala, India is reported. The major industries operating near the site were identified. The samples were collected from the site according to the guidelines provided by United States Environmental Protection Agency. The samples collected from the site as well as the industrial outlets were analysed to check the presence of heavy metals and organic compounds. The concentration of heavy metals and organic compounds at the contaminated site were higher than the corresponding background soil concentrations indicating the site to be highly contaminated. The presence of DDT (Dichlorodiphenyltrichloroethane) was found in the samples from the site. Iron, Nickel, Copper, Zinc, Chromium and Lead were found in the site as well as in the outlets of the industries, indicating that they may be contributed by any of the identified industries. Similarly, Endosulfan and HCH (Hexachlorocyclohexane) was found at the site. The sampling results of site were also compared with world shale average. All contaminants except for Zinc and Lead were lower than the world shale average. The extent of contamination and possible sources of contamination are identified. The results of the investigation can be used for assigning remediation liability among the polluters.

#### **4.10.T-01 The Application of the Precautionary Principle in European Union Waste and Chemicals Regulation From a Material Circulation Perspective**

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Both the EU waste and chemicals regulations are based on the precautionary principle (PP). The main objective of the chemicals regulation is to protect human health and the environment from the risks posed by hazardous chemicals. The modern waste regulation promotes, among other things, the recovery of waste. These material circulation aims have recently been reinforced by the introduction of ambitious circular economy (CE) targets. Balancing between the material circulation objectives and the safety objectives is the main dilemma of the precautionary regulation of the material circulation.

We examined the role of the PP in balancing the material circulation objectives and the safety objectives in EU waste and chemicals regulation. We analysed the relevant case law, discussed the role of scientific uncertainty in the precautionary regulation of risks, and proposed a classification of risk problems in the CE.

The European Court of Justice (ECJ) and the General Court have applied the PP in several cases concerning restrictive measures on hazardous chemical substances. This has led to, for example, allowing the identification of substance as a Substance of Very

High Concern under REACH in the existence of uncertainties as regards the determination of safe level of exposure to that substance. In waste regulation, the ECJ has pointed out that special precaution must be taken as regards the potentially hazardous nature of the production residues that are intended to be recovered.

Application of the PP involves deliberation on a range of normative dimensions, for example, the determination of the chosen level of protection. Risk problems of the CE, classified as simple, complex, uncertain, ambiguous and purely hypothetical, also include diverse values on how to deal with a specific risk. The PP does not apply to purely hypothetical risks, nor does it apply to simple risk problems. Our analysis highlighted uncertainties related to decision-making in ambiguous risk problems. They may result from complexity or weak knowledge about the underlying phenomena, leading to interpretative ambiguity, but also to the need to balance different values (normative ambiguity) and environmental impacts in the decision-making.

The dilemma can only be solved by balancing risks and benefits and reconciling different objectives through an analysis that gives due weight to different types of risk problems and their associated uncertainties and ambiguities in an inclusive discourse.

#### **4.10.T-02 Uncertainty and precaution in waste management: experimental assessment of the variability generated by waste sampling for classification and disposal**

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In the field of waste management, decisions made upon uncertain characterization data could lead the involved actors to be improperly charged, e.g., for incorrect waste classification. This forces waste managers to adopt overprecautionary measures influencing significantly the economic sustainability of their projects. To address this trend, in determining whether legal limits are exceeded, environmental forensics should take into account the data uncertainty upon which decisions are made. Uncertainty can be quantified by the variability (i.e., variance) of data generated by the materials characterization process. It is now recognized that this variability is mainly determined by the performed sampling procedures, while a minor role is played by the consolidated analytical techniques. A powerful tool to assess the total variability is represented by the so-called Replication Experiments (RE). By replicating the sampling performance in “fully nested experiments”, RE allow to calculate the value of the generated total variance together with its components (i.e., sampling and analysis), through nested ANOVA performed according to the applied experimental design. Whether these experiments are often included in QA/QC procedures for goods production, no examples can be found in the scientific literature about the application of RE on waste characterization.

As first of his genre, this work shows and discusses the results of a RE applied to evaluate the efficiency of the sampling phase defined within a characterization plan aimed at classification of mineral industrial waste. The case history is related to a remediation and disposal, funded by Veneto Region, of about 44,000 tonnes of waste of unknown origin, illegally stored in a 11,200 m<sup>2</sup> former industrial building located in the province of Padova, NE Italy.

The total variability of the majority of the investigated parameters was generated by spatial variability, thus supporting the criteria applied for the classification of waste in different, spatially grouped, lots. Sampling variability contributes could be acceptable for most parameters measured in the solid samples with one exception (Mn), while eluates showed rather higher values, mainly in terms of DOC.

These results provide forensic scientists a scientifically sound tool to better interpret possible non compliance judgments, based on single concentration values, derived from the analysis of the materials investigated during subsequent investigations.

#### **4.10.T-03 Vinyl Chloride Enhances High Fat Diet-Induced Proteome Alterations in the Mouse Pancreas Related to Metabolic Dysfunction: Implications for Individual Susceptibility**

**Yue Ge, Maribel Bruno, Maliha Nash and Brian Chorley, U.S. Environmental Protection Agency**

Alterations in physiological processes in pancreas have been associated with various metabolic dysfunctions and can result from environmental exposures, such as chemicals and diet. It was reported that environmental vinyl chloride (VC) exposure, a common industrial organochlorine and environmental pollutant, significantly exacerbated metabolic-related phenotypes in mice concurrently fed high-fat diet (HFD) but not low-fat diet (LFD). However, little is known about the role of the pancreas in this interplay, especially at a proteomic level. The present study was undertaken to examine the protein responses to VC exposure in pancreas tissues of C57BL/6J mice fed LFD or HFD. We demonstrated that VC-induced pancreas proteome changes were largely linked to metabolic processes in the pancreas that can contribute to diabetes and other facets of metabolic syndrome, such as insulin production and beta cell proliferation e.g., decorin, insulin-like growth factor-1, periostin, phosphorylated protein kinase B (pAKT) and phosphorylated glycogen synthase kinase 3  $\beta$ . In addition, pancreatic proteins altered by HFD were also augmented by VC exposure compared to mice fed LFD. This result suggested that VC exposed mice might be more sensitive to adverse impacts of HFD. These data suggest that environmental toxicant exposures, such as occupational exposures to VC, may lead to individual susceptibility to adverse metabolic consequences in the pancreas due to dietary choices.

#### **4.10.T-04 Characterizing Genetic Susceptibility in Populations Vulnerable to Pesticide Exposures**

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Interactions between environmental factors and genetics underlie the majority of chronic human diseases. However, connecting chemical exposures and genetic susceptibility to assess how chemicals exert variable effects in a population is a challenge. Therefore, new approach methodologies (NAMs) are necessary to determine the role of genetic variability in chemically-induced adverse outcomes to protect vulnerable populations in chemical risk assessment. To assess molecular mechanisms of individual susceptibility to chemicals, we formed a dataset of chemicals, points of genetic variability (single nucleotide polymorphisms,

SNPs), and diseases using toxicity pathways. These Pesticide-SNP-Disease linkages describe genetic variants that chemicals may act on to lead to variable outcomes in a population. A geospatial analysis was done at the country level globally and refined to the county level in the United States (US) using pesticide application data for 491 chemicals. Based on where pesticides were applied, we identified SNPs implicated by those pesticides in our dataset. Many SNPs were implicated by multiple pesticides meaning these SNPs may represent important points for genetic susceptibility to adverse outcomes in the general population. While many of the same diseases were implicated by multiple pesticides, we found that different SNPs were implicated in different diseases. For example, of the 149 SNPs cypermethrin implicated in Alzheimer's Disease and Parkinson Disease, 80% were only implicated in one disease and not the other. Further, the toxicity pathways implicated by cypermethrin differ. This describes how, depending on the SNPs present in an individual, different diseases may develop following the same pesticide exposure. Therefore, using our dataset, SNPs and toxicity mechanisms important in individual susceptibility to specific diseases can be prioritized in different geographic regions based on pesticide use. Finally, we assessed our results against US disease incidence reported in the Global Burden of Disease. For Alzheimer's Disease and Parkinson Disease, we found that more SNPs implicated in our dataset correlated to greater disease incidence reported in a US state. While many exposures likely contribute to disease outcomes, our framework represents a promising NAM to predict genetically vulnerable populations in different regions based on where chemicals are used to help inform individual susceptibility in human health risk assessment.

#### **4.10.P New Approaches, Methodologies and Policies in Environmental and Human Health Risk Assessment**

##### **4.10.P-Tu357 Exploring the Correlation Between Anthropogenic Contamination, Methylation Variation, and Adverse Health Effects in Olfactory Bulb Tissues**

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Introduction of anthropogenic contaminants (which include pollutants such as smoking, microplastics, and occupational pollution) into the body has called for more study on the biological effects of this exposure. Inhalation, a potential entrance pathway for pollution, can damage tissues in the olfactory system and the brain, leading to potential neurodegeneration. Contaminant exposure can affect many biological processes in the cells of these tissues, one of these being epigenetic modification through methylation. Methylation is a modification of DNA which plays a role in gene expression and cell differentiation. Variation of this process is evidenced to cause a variety of adverse health outcomes such as cancer and neurodegeneration. There are a variety of factors that are linked to influencing these epigenetic changes, including exposure to aforementioned contaminants and medical comorbidities. However, little is known about the relationship between anthropogenic contamination and epigenetics in olfactory bulb tissues, and the significance of this relationship to neurodegeneration. This study aimed to investigate the relationship between anthropogenic contamination exposure and methylation variation in olfactory bulb tissue. The overarching hypothesis for this work was that patients with greater occupational or residential exposure to these environmental contaminants will show altered methylation signatures as compared to controls. Olfactory bulb tissue samples from individuals with diverse demographic and occupational backgrounds and associated epidemiological data were provided by a partnering university and pre-classified as either cancerous or healthy. The samples are dissociated and DNA is extracted. DNA concentration is quantified and each sample's methylation signature is profiled using Infinium MethylationEPIC BeadChip array on Illumina. These signatures are then referenced with the aforementioned background of each individual to explore any potential correlation. This exploratory study aimed to investigate the relationship between environmental exposures to anthropogenic chemicals and their impact on adverse human methylation signatures. Such a correlation could shed light on the long-term effects of environmental contamination on olfactory tissue, specifically through the epigenetic and neurodegenerative lenses. Anthropogenic contamination concerns could encourage early screenings and detection for neurodegeneration and other diseases.

##### **4.10.P-Tu359 Geographic Disparity in Asthma Hospitalizations: The Role of Environmental Air Pollution**

**Sonia Munoz and Carlos Mazariegos**, *Environmental Toxicology, Texas Tech University*

Public community health is an to community air quality. Annually, costs from air pollution-related illnesses are estimated at \$150 billion and result in more than 100,000 premature deaths in the United States alone. The spatial distribution of Lubbock County, Texas, United States, is associated with hospitalizations caused by asthma due to environmental pollution factors. This current dynamic creates geographic disparities, specifically among industrial and residential sites. The proximity of residential areas to manufacturing sites is associated with an increased risk of respiratory distress due to airborne toxins. The population health related to asthma in Lubbock County is documented in public health records, thus serving as an indicator of the local environmental air pollution. Characterization and regulation of human and environmental health criteria are identified by the United States Environmental Protection Agency (US EPA) criteria for air pollutants. These pollutants are crucial for the determination of permissible pollutant levels. This study will utilize the Emission Weighted Proximity Model (EWPM) to measure the total quantities of pollutants from industrial facilities within Lubbock County and correlate the results to the Toxic Release Inventory (TRI) to investigate the line exposure of off-target residential areas in Lubbock County. rrent analyses are underway. Environmental air pollution, as a neighborhood-level factor, must be studied and evaluated to properly characterize and assess pollution-related hospitalizations related to asthma within the community. Exposure assessment is needed to address and minimize these hospitalizations related to asthma. Reduction of air pollution is a key foundation of economic growth, public

health awareness, and the future development and well-being within the community. Additionally, the reduction and prevention of air pollution is vital to the conservation of air as a natural resource.

#### **4.10.P-Tu360 Morphological Transformation and Phenotypic Plasticity of Normal Human Breast Epithelial Cells after 40-Week Exposure to Low-Dose Cadmium**

**Katelyn M. Polemi, Anagha Tapaswi, Jade Schroeder, Rachel K. Morgan, Laurie K. Svoboda, Dana C. Dolinoy and Justin A. Colacino, University of Michigan, Ann Arbor**

As of 2021, breast cancer is the most commonly diagnosed cancer worldwide with the 5th highest mortality rate of any cancer in the US. Breast tumors are highly heterogeneous resulting from the acquisition of morphological alterations and cancer hallmarks including stemness and cellular plasticity. Roughly 80-85% of cases occur in women with no family history of the disease indicating how heavily lifestyle and environmental factors play in the disease. Cadmium (Cd) is a naturally occurring toxic heavy metal and a known lung carcinogen, however, its role in breast cancer remains controversial. *In vitro* studies have shown that breast cells exposed to Cd are malignantly transformed through estrogen receptor independent mechanisms. Here we investigate the role of long term (40-week) low dose Cd (0.25uM and 2.5uM) exposure on cancer-associated morphological alterations and cellular plasticity. Cell Painting, a high content image-based assay for morphological profiling, was used to measure cellular morphology of the cells in an unbiased manner every 10 weeks starting with week 0 through week 40. Hoechst, Concanavalin A, Syto14, Phalloidin, MitoTracker, and Wheat Germ Agglutinin (WGA) were used to stain DNA, endoplasmic reticulum, cytoplasmic RNA, actin, mitochondria, and golgi/plasma membrane, respectively, and fluorescence intensity was quantified using CellProfiler software. Using immunofluorescence, we also quantified expression of Keratin 8 and Keratin 14, markers of luminal and basal cells, respectively in treated and control cells. During image processing, the nuclear-cytoplasmic ratio and nuclear size were each separately categorized as “high” if it exceeded the 90<sup>th</sup> percentile among all cells imaged and all others categorized as “low”. Within the population of cells exposed to 2.5uM Cd for 30 weeks, there are 3-times the number of cells categorized as having a higher than average nuclear-cytoplasmic ratio compared to the control. Additionally, week 30 cells exposed to 2.5uM Cd were 3-times more likely to be in a higher category for nuclear size. Studies have shown that an increased nuclear-cytoplasmic ratio and larger nucleus have been associated with breast malignancy. After 36 weeks of 2.5uM Cd exposure, Keratin 14 intensity significantly decreased compared to control. Our analysis shows transformation of MCF10A cellular morphology at week 30 with the physiologically relevant dose of Cd exposure 2.5uM.

#### **4.10.P-Tu361 Application of Precautionary Principles in Environmental Crime Reconstruction**

**Bindu A. Gopalakrishnan<sup>1</sup> and George Kuttiparichel Varghese<sup>2</sup>, (1)Civil Engineering, National Institute of Technology Calicut, India, (2)National Institute of Technology Calicut, India**

In the analysis of environmental crimes, scientific uncertainty is a key factor preventing concrete actions for protecting the environment. However, the adoption of precautionary principle requires taking preventive action in the face of scientific uncertainty. It also involves a shift in the “burden of proof” to the proponent of a project if there is an adverse environmental consequence. This study explains how Crime Reconstruction (CR) technique, involving the steps of recognition, identification, individualization and reconstruction, can be employed in environmental crimes and how, along with the conventional steps of crime reconstruction, the elements of precautionary principle can be applied in the various stages of reconstruction.

While giving clearance for a project, conditions are specified by the granting authority on the type and quantity of polluting emissions permitted. A violation of this condition constitutes an offence, and can be *recognized* as an evidence of the crime considering the precautionary principle. In the *identification* stage of CR, the characteristic properties of an item recognised is compared with that of the standard objects in order to classify them. In a multi-party pollution case, the violation of the emission standards by any unit is an indication of its involvement in crime. The characteristics of the pollutant exceeding the limits also helps in apportioning the criminal liability.

The releases from every industry will surely have its own signature components which makes it different from others. Review of the environmental clearance documents detailing the raw materials of production and their sources along with the processes of the industry will give some clue about the possible signature evidences. If it is able to find out that evidence among the identified set of evidences, the role of a particular industry can be *individualised* and their relative contribution to the pollution scenario can be assessed more precisely. The identification and individualization analyses of physical evidence and the conclusions drawn from them are important ingredients in a final reconstruction. In this stage, a set of actions such as data collection, conjecture, hypothesis formulation, its testing and theory formation are to be performed to achieve a successful reconstruction. Here a critical analysis of the deviations from the approved environmental clearance conditions will be useful.

#### **4.10.P-Tu362 Investigating Chemical Exposures as Drivers of Phenotypic Plasticity in Normal Human Breast Tissue via High Content Imaging**

**Jade Schroeder, Anagha Tapaswi, Katelyn M. Polemi and Justin A. Colacino, University of Michigan, Ann Arbor**

Breast cancer is the most diagnosed cancer, as well as the primary cause of cancer death in women worldwide. Of the different breast cancer subtypes, triple negative breast cancer (TNBC) is particularly aggressive and is associated with poor prognosis. Black women are two to three times more likely to be diagnosed with TNBCs than white women. BRCA1 mutations are thought to be drivers of 10-20% of TNBC, however etiological drivers remain unclear for women without these polymorphisms. Recent experimental evidence suggests that basal-like TNBCs may derive from luminal cells which acquire basal characteristics through phenotypic plasticity, a new hallmark of cancer. Whether chemical exposures are drivers of phenotypic plasticity in breast cancer is poorly understood. To address this research gap, we developed a high content immunocytochemistry assay using normal human breast cells to test whether chemical exposures can impact luminal/basal plasticity by unbiased quantification of keratin 14



(KRT14), a known basal-myoepithelial marker; keratin 8 (KRT8), a known luminal-epithelial marker; Hoechst 33342, a DNA marker; and phalloidin, a marker of the cell cytoskeleton. A cell line established from healthy tissue from a donor to the Susan G. Komen Normal Tissue Bank was exposed for 48 hours to three different doses (0.1  $\mu$ M, 1  $\mu$ M, and 10  $\mu$ M) of eight ubiquitous chemicals (Arsenic, BPA, BPS, Cadmium, Copper, DDE, Lead, and PFNA) with documented exposure disparities in US Black women in triplicate. Automated fluorescence image quantification was performed using Cell Profiler software, and a random-forest classifier was trained to classify individual cells as KRT8 positive, KRT14 positive, or hybrid (both KRT8 and KRT14 positive) using Cell Profiler Analyst. We saw significant dose-dependent increases of hybrid populations in response to BPA as well as low dose effects of PFNA. The increase in hybrid populations expressing both KRT14 and KRT8 is indicative of a phenotypically plastic progenitor-like population in line with known theories of carcinogenesis. Work is ongoing to test effects in cell lines from diverse individuals to identify consistent trends or interindividual differences in response. These results further elucidate the relationship between chemical exposure and breast phenotypic plasticity and highlight potential environmental factors which impact TNBC risk.

#### 4.10.P-Tu363 Monitoring of Insect Traces in Japanese Bioaerosols using NGS

**Hitoshi Iwahashi Dr.**, Gifu University, Gifu, Gifu, Japan

Studies on bioaerosols have primarily focused on their chemical and biological compositions and their impact on public health and the ecosystem. However, most bioaerosol studies have only focused on viruses, bacteria, fungi, and pollen. To assess the diversity and composition of airborne insect material in particulate matter (PM) for the first time, we attempted to detect DNA traces of insect origin in dust samples collected over a two-year period. These samples were systematically collected at one-month intervals and categorized into two groups, PM<sub>2.5</sub> and PM<sub>10</sub>, based on the aerodynamic diameter of the aerosol particles. Cytochrome-c oxidase I (COI) was the barcoding region used to identify the origins of the extracted DNA. The airborne insect community in these samples was analyzed using the Illumina MiSeq platform. The most abundant insect sequences belonged to the order Hemiptera (true bugs), whereas order Diptera were also detected in both PM<sub>2.5</sub> and PM<sub>10</sub> samples. Additionally, we inferred the presence of particulates of insect origin, such as brochosomes and integument particles, using scanning electron microscopy (SEM). This provided additional confirmation of the molecular results. In this study, we demonstrated the benefits of detection and monitoring of insect information in bioaerosols for understanding the source and composition. Our results suggest that the PM<sub>2.5</sub> and PM<sub>10</sub> groups are rich in insect diversity. Lastly, the development of databases can improve the identification accuracy of the analytical results.

#### 4.10.P-Tu364 NGS analysis of biological traces extracted from bioaerosols

**Junko Takahashi**, Graduate School of Information, Production and Systems, Waseda University, Japan

Bioaerosols are atmospheric particles with a biological trace, such as viruses, bacteria, fungi, and plant material such as pollen and plant debris. In this study, we analyzed the biological information in bioaerosols using next generation sequencing of the trace DNA. The samples were collected using an Andersen air sampler and separated into two groups according to particulate matter (PM) size: small (PM<sub>2.5</sub>) and large (PM<sub>10</sub>). Amplification and sequencing of the bacterial 16S rDNA gene, prokaryotic internal transcribed spacer 1 (ITS1) region and DNA sequence of a plant chloroplast gene (*rbcL*) were carried out using several sets of specific primers targeting animal and plant sequences. Lots of bacterial information was detected from the bioaerosols. The most abundant bacteria in several samples were of the Actinobacteria (class), Alphaproteobacteria, Bacilli, and Clostridia. For the animal detection using internal transcribed spacer 1, only uncultured fungi were detected in more than half of the hits, with a high number of *Cladosporium* sp. in the samples. For the plant identification, the ITS1 information only matched fungal species. However, targeting of the *rbcL* region revealed diverse plant information, such as *Medicago papillosa*. In conclusion, traces of bacteria, fungi, and plants could be detected in the bioaerosols, but not of animals using our primers.

#### 4.10.P-Tu365 Soil's Organic Carbon Influence on the Dermal In Vitro Bioavailability of Parent and Alkylated PAHs in Former Gasworks Soils

**Alison Williams-Clayson<sup>1</sup>**, Chris Vane<sup>1</sup>, Darren Beriro<sup>1</sup>, Matthew Jones<sup>2</sup> and Russell Thomas<sup>3</sup>, (1)British Geological Survey, United Kingdom, (2)University of Nottingham, United Kingdom, (3)WSP, United Kingdom

Polycyclic aromatic hydrocarbons (PAH) pose a potential risk to human health, specifically when former contaminated industrial sites are reused to create places for people to live and work. Dermal contact with contaminated soil is one of the exposure pathways, and accounts for 5.5% of the total exposure in a residential site. The estimated risk is calculated using 13% of the total concentration in deterministic exposure models, this value is uncertain and is based only on benzo[a]pyrene (B[a]P). PAH are complex mixtures, many of the individual compounds behave in different ways to B[a]P, in particular the behaviour of alkylated PAH is unknown. Alkylated PAHs are potentially a greater problem on sites where oil is the main contamination source, studying their behaviour alongside parent PAH in real-world samples means that this research can relate to a wider range of contamination scenarios.

This research investigates 30 parent PAH and 21 alkylated PAH *in vitro* dermal bioavailability against selected soil properties from Rock-Eval(6) pyrolysis and particle size analysis to understand factors affecting parent and alkylated PAH release from former gasworks soils. The *in vitro* dermal bioavailability of 51 PAH were calculated from dermal diffusion cell experiments. Extractions of the applied soil, artificial skin, and receptor solution of five gasworks soils at three-time steps were quantified using GC-MS/MS. Total concentrations of B[a]P in these five soils ranged from 13.54 to 160.49 mg/kg, above the generic assessment criteria for residential land use. The samples include characterised soil reference material and real-world samples from a range of gasworks sites where different processes have operated and a detailed understanding of the site history has been obtained. Dermal bioavailability data will be presented to show differences between parent and alkylated PAH, this data will be

compared with physical and chemical properties to determine tentative cause effect relationships affecting the release individual compounds. This research is important to landowners, remediation companies, consultants, and regulators as it provides a new line of evidence for reducing uncertainties in site specific risk assessment of the chronic exposure of carcinogenic chemicals in soils on previously developed 'brownfield' land.

#### **4.10.P-Tu366 BMD Analysis of Multiple Endpoints in Human Health Risk Assessment: Current Practice and Challenges** *Lily Wang, Todd Blessinger, Allen Davis and Jeff Gift, U.S. Environmental Protection Agency*

Human health risk assessment often evaluates multiple endpoint variables in order to form a comprehensive picture of the toxicity effects of an environmental chemical or toxicant. Benchmark dose (BMD) modeling is a flexible method that takes the shape of the dose-response curve and important measures of uncertainty and variability in the data into account. While BMD modeling is an improvement over previous (e.g., NOAEL) methods, existing BMD models are designed to evaluate single or independent endpoints, not multiple related endpoints simultaneously. Therefore, development of an advanced BMD approach for efficient analysis of multiple related endpoints could be beneficial to risk assessment. This presentation will present our research in univariate, ordinal and multivariate analysis of multiple endpoints including simultaneous analysis of multiple correlated endpoints using environmental chemical toxicity data. Our results demonstrate that multivariate BMD approaches produced comparable and stable BMDLs in a single analysis, with less variability and more robust estimation of adverse health outcomes. In addition, this presentation will also include the major approaches that are currently used in multivariate modeling of multiple endpoints for human health risk assessment, and the challenges in applying these models, such as model choice and model averaging, clustered, Bayesian versus likelihood or frequentist methods, and the analysis of aggregate versus individual data. *(The information in this Abstract has been subjected to review by the Center for Public Health and Environmental Assessment and approved for presentation. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.)*

#### **4.10.PC New Approaches, Methodologies and Policies in Environmental and Human Health Risk Assessment**

##### **4.10.P-Tu358 Measurement and modelling strategies for a study of health effects caused by urban ultrafine particle concentrations**

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Ultrafine particles (UFP,  $D_p < 100$  nm) can be formed directly e.g. by combustion processes or secondarily in the atmosphere by mostly photochemical processes from gaseous precursors. Due to their small size, they can penetrate deep into the lung and reach the alveolar region. From there, they may be translocated into other organs, where they may cause inflammatory reactions. It is assumed that organ damage and chronic diseases e.g. of the lung and the cardiovascular system or a weakening of the immune system are promoted by persistent inflammations. However, the health effects of UFP have not yet been fully elucidated.

Knowledge gaps concerning long-term effects of UFP exposure are mainly due to the scarcity of corresponding epidemiological studies, which in turn are a result of the lack of high-resolution UFP concentration maps.

Furthermore, the effect of UFP has to be separated from that of other pollutants that are partly correlated with UFP like PM<sub>10</sub>, NO<sub>2</sub> or Black Carbon concentrations as well as noise has not yet been conclusively clarified.

The project KoPilot aims at filling the knowledge gap by designing measuring and modelling concept for an epidemiological study. The goal of KoPilot is to develop and experimentally test a measurement and modelling strategy for UFP that allows for the distinction of health effects of UFP and the abovementioned co-exposures. Modelling and measurement activities in this pilot study will focus on a limited urban area within Düsseldorf, Germany.

The first question to be answered is how the UFP concentration data, required for epidemiological studies of long-term exposure, can be determined accurately and validly so that personal exposure can be approximated as closely as possible. The second question is how effects from UFP can be separated from other confounders.

The measuring and modelling concept will be presented and discussed at this conference in view of their validity and knowledge gaps for future epidemiological studies.

#### **4.10.V New Approaches, Methodologies and Policies in Environmental and Human Health Risk Assessment**

##### **4.10.V-01 Association Between Mercury Levels and Human Reproductive Health: A Cross-Sectional Study**

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Infertility is defined as a disease of the reproductive system characterized by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility rates have decreased worldwide, and some studies showed that lifestyle and environmental risk factors (e.g., exposure to chemicals) may be responsible for this decline. Mercury (Hg) is one of the most prevalent contaminants in the environment, generated by anthropogenic activities. Hg exposure has been associated with human reproductive health decline since subjects with unexplained infertility had higher total Hg (THg) levels in hair, blood,

and urine than fertile ones and high THg levels were associated with longer time-to-pregnancy and adverse reproductive outcomes such as spontaneous abortions, and increased rates of menstrual disorders. Our main goal was to evaluate if Hg exposure affects the reproductive health of cohort from the Aveiro region (Portugal) using non-invasive matrices. For that, eligible women and men were recruited at Centro Hospitalar do Baixo Vouga (Aveiro, Portugal). A detailed questionnaire regarding sociodemographic, diet, lifestyle and reproductive data was completed, and samples of hair, saliva, urine, semen, placental tissue, and umbilical cord were collected. Semen samples were processed according to World Health Organization standards. THg levels in hair from both men and women, as well as in placental and cord tissues samples were quantified by atomic absorption spectrometry after thermal decomposition of the sample using the Advanced Mercury Analyzer (AMA-254, LECO), while urine metabolomic profiling was performed by nuclear magnetic resonance (NMR) spectrometry. Our study demonstrated Hg bioaccumulation in biological samples from male and female participants living in the Aveiro region. A significant positive correlation was found between THg levels in hair and all matrices analysed, reinforcing the feasibility of the use of human hair to assess Hg exposure. Moreover, significant positive correlations between THg levels in men's hair and the percentage of sperm tail defects and the teratozoospermia index were found. Regarding metabolomic analysis, we showed that lifestyle and Hg exposure modifies several metabolites related with glycolysis metabolism. Finally, this study demonstrated that lifestyle habits seemed to play a role in Hg exposure, but further investigations are necessary.

#### 4.11 Progress in Knowledge of Rare Earth Elements

##### 4.11.P-Tu367 Rare Earths and Yttrium in Tissues and Shells of Bivalves from the Danube River and its Tributaries

**Keran Zhang<sup>1</sup>, Anna Lena Zocher<sup>2</sup> and Michael Bau<sup>2</sup>,** (1)CritMET – Critical Metals for Enabling Technologies, School of Science, Constructor University Bremen, Germany, (2)CritMET – Critical Metals for Enabling Technologies, School of Science, Contractor University Bremen, Germany

Due to their numerous applications in various high-technology products and processes, Rare Earths and Yttrium (REY) have become microcontaminants in freshwater systems. Recent toxicological studies demonstrated that REY represent toxicities for aquatic organisms under laboratory conditions. Nevertheless, their biogeochemical behaviour, especially their uptake by aquatic organisms is still poorly understood.

Here, we report on the concentration and distribution of REY in the shells of four different mussel species, in the tissue and shells of three *Anodonta anatina* mussels, and in the respective ambient waters from the Danube River and some tributaries. The shells were grouped according to their sizes and then meticulously cleaned, while the soft tissues were dissected and lyophilized. All samples were acid-digested and preconcentrated prior to ICP-MS measurements. Analytical quality was monitored by analysing the certified reference materials JLS-1 and BCR-668 for shells and tissues samples, respectively.

Size dependency of the REY concentration in shells was only observed for *Corbicula* shells, corroborating previous observations from the Rhine River. Shale-normalized REY patterns are similar for all shells and tissues, and they are slightly middle REY-enriched. Compared to the respective 0.2 µm-filtered ambient water, the biological samples show REY concentrations that are between three to five orders of magnitude higher, revealing significant bioaccumulation of REY. The observed preferential uptake of light REY relative to heavy REY is due to more stable complexation of HREY with dissolved ligands. Despite significant anthropogenic Gd enrichments in ambient water, there are no positive Gd anomalies in the biological samples, suggesting biounavailability of the Gd-based contrast agents present in the waters of the Danube River.

##### 4.11.P-Tu368 Toxicity of REE Along the Food Chain Microalgae-Daphnia in a 14-Day Nanocosm

**Chantal K.E. van Drimmelen<sup>1</sup>, Andrew Hursthouse<sup>2</sup> and Susanne Heise<sup>3</sup>,** (1)Ecotoxicology, University of Applied Science Hamburg, Germany, (2)University of the West of Scotland, United Kingdom, (3)Hamburg University of Applied Sciences, Germany

While it is impossible in the lab to reflect the variability of responses and extent of interactions in natural systems to e.g. contaminant stressors, cosm-experiments can be set up that feature more than one species and run longer compared to single species bioassays. In order to find out, how rare earth elements (REE) impact the dynamics of organisms nanocosms were set up featuring two biological species from two trophic levels: the water flea *Daphnia magna* and its food organism, the microalgae *Raphidocelis subcapitata*. Our nanocosms consisted of PET-cups with characterized sediment (Lufa 2.2) and M4 media without EDTA. They were spiked with environmentally and anthropogenically relevant nominal concentrations of La or Gd (1, 10, 100 and 1000 µg L<sup>-1</sup>) and ran for 14 days. The experiment was repeated at least 5 times. Monitored endpoints comprised parameters such as death rate, reproduction rate, and population count, according to the guidelines of the single-species tests. Our hypothesis was that reproduction of *D. magna* would be a more sensitive endpoint after REE exposure compared to growth rate of *R. subcapitata*, and that the heavy REE gadolinium would be more toxic than lanthanum, in agreement with previous studies we had done. The nanocosms were analyzed over a course of 14 days using a sacrificial method. Preliminary results indicated that differences between effects under La and Gd exposure were not significant, probably due to the high variability in the nanocosms. In detail, reproduction of the daphnia was not significantly affected by the REE, meaning that this part of the hypothesis can be falsified. There was however, a clear trend for a delay of the onset of reproduction of daphnids between the control on one side, and La and Gd on the other. The growth rate of the algae also appeared to be impacted by REE. Analysis of data is being carried out and will be presented during the conference.

##### 4.11.P-Tu458 Assessing Individual and Ternary Mixture Ecotoxicity of Three Rare Earth Elements for Tomato and Durum Wheat in Soil, using Total, Bioaccessible and Tissue Concentrations as Dose

**Taylor A Vanderyagt and Beverley A. Hale,** University of Guelph, Canada

Rare earth elements (REEs) comprise 17 elements of the lanthanide series plus Yttrium and Scandium, found in minerals worldwide in a range of concentrations. Due to similar physicochemical properties including luminescent and catalytic features, REEs have become crucial to a wide range of modern technologies such as green energy applications, hybrid vehicles, manufacturing industries; all accelerating in demand on a global scale. Despite increasing exploitation and documented REE pollution, REEs remain data-poor metals with concerns raised about their toxicological potential especially for terrestrial environments. For singular exposures, toxicity has been determined for some REEs but quantitative understanding of mixture ecotoxicity and interactions of REEs is limited. The objective of this work was to determine toxicity thresholds for terrestrial plant endpoints for singular and ternary mixture REE exposures, using low effect levels most relevant to risk assessment (EC10, EC20) and two agricultural species (Tomato and Durum Wheat). Toxic effect concentrations for tomato and durum wheat were determined for three REEs, Cerium, Neodymium, and Europium, in singular and ternary mixture exposure doses. Singular thresholds revealed Ce as the most toxic element, with all REE thresholds (EC10) ranging from 23- 596 mg/kg<sup>-1</sup> based on total soil concentrations. Assuming concentration addition (CA), ternary mixture exposures were found to be both less than and more than additive among the 18 combinations of species and endpoint per the three REEs, compared to the sum of toxic units (ΣTUs) derived from single metal thresholds (“toxic units”). This was true whether dose was expressed as total or bioaccessible soil REE concentrations. However, when REE dose was expressed as internal concentrations (tissue), ternary mixture endpoint responses were found to be more similar to that of singular exposures, i.e. responses were additive or slightly less than additive. Internal tissue concentrations may serve as a better predictor of toxicity compared to external concentrations for both singular and mixture exposures, in that internal concentrations reveal at which level of plant exposure (soil, plant interface with soil, biotic ligand) REE interactions occur.

#### 4.11.T-01 Rare Earth Elements binding to goethite: experimental and modeling investigations

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Rare Earth Elements (REE) are not only considered as high-technology components but also as emerging contaminants when released into ecosystems. However, there is a lack of comprehensive investigations on their mobility, environmental fate and transport in geochemical settings. REE can bind to the surface of minerals such as goethite, which can alter their fate and transport. Here, we have thoroughly examined the adsorption of the entire REE group with goethite via batch experiments at environmentally relevant geochemical conditions, over a wide range of pH and varying REE concentrations. As typically encountered for cations, REE binding increased with increasing pH values. Results from the experiments showed that at low pH values there is a stronger adsorption for middle REE (MREE) as compared to light REE (LREE) or heavy REE (HREE). Conversely, at higher pH values HREE are preferentially complexed. These two distinct complexation processes likely arise due to two different types of binding sites: (i) a larger number of weak sites that prefer HREE occurring on the major faces of goethite particles, and (ii) a smaller number of strong sites that primarily complexed MREE and occur at the termination of the goethite particles. A Surface Complexation Model (SCM) based on CD-MUSIC approach has been developed for goethite, which successfully predicted both sorption edges and sorption isotherms. These results have strong implications for an accurate assessment of the transport behavior of REE in natural systems.

#### 4.11.T-02 How does the pH-dependency of the Colloid Structure Affect Rare Earth Elements Mobility in the Environment?

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Rare earth elements (REE) are used extensively for the manufacture of high-tech products, which drives up demand for and spread of these elements. As a result, questions have been raised about their impact on the environment. The ability of rare earth elements to bind to solid surfaces, particularly colloids is expected to mainly controls the REE mobility and transport between the various environmental compartments. Given their ubiquity and the rise of their concentration in natural in response to climate change, we are particularly interested in colloids composed of organic matter (OM) and iron (Fe). Recent research revealed that they are made up of Fe nano-oxides fractally organized into primary and secondary aggregates complexed with OM molecules or linked to larger OM colloids. The reactivity of these colloids is governed by this organization and the resulting surface site availability. In this study, our objective is to investigate how pH affects the binding of REEs by these colloids and their subsequent mobility in the environment. The Fe-OM colloids were synthesized by OM titration with Fe(II) solution at pH 6.5. Rare earth elements (whole group, from La to Lu) adsorption experiment by colloids were performed on a range of REE/Fe molar ratios at pH 6 and 4 and ionic strength of 0.05 mol L<sup>-1</sup> (NaCl).

The results demonstrated that REE are mainly bound to the OM part of the Fe-OM colloids and that their mobility is controlled by the OM response to the pH conditions. At pH 6, small organic molecules are solubilized with their REE loading, REE mobility is no more controlled by the Fe-OM colloids mobility but also by the strong solubilization of these small organic molecules. By contrast at pH4, Fe-OM colloids bound lower amount of REE (4.4 mg g<sup>-1</sup> against 12 mg g<sup>-1</sup> at pH 6), but aggregated. The major part of REE stay soluble in solution but that bound to Fe-OM colloids is expected to be immobilized by the colloid settlement or by the colloids trapping in soil and sediment porosity.

**Keywords:** Rare earth elements, Organo-mineral Colloid, colloidal structure , pH dependency , Adsorption

#### 4.11.T-03 Effects of Sub-Chronic Exposure of Model organism *Daphnia magna* to Rare Earth Elements La and Gd

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Rare earth elements have become a vital resource in the technology we use in our everyday life. The increase in their use has led to studies focusing also on their effects on the environment, recognized as emerging contaminants. Many studies have focused on the acute effects of these elements on model organisms, however, one of the challenges remains in simulating a more realistic exposure that can give more information about the sub-lethal effects. For this reason, a non-standard test design was elaborated where the model organism *D. magna* was exposed for 7 days to two rare earth elements La and Gd to assess their individual toxicity.

The aim was to assess the mortality and sub-lethal effects such as feeding rate, somatic growth, and maturity. Test organisms were exposed to a series of concentrations ranging from 0.5 to 5 mg/L for both elements and fed daily with green algae *Raphidocelis subcapitata*.

The results obtained for the mortality are expressed as the daily LC<sub>50</sub> values, which decreased with the increasing length of exposure. The value for La ranged from 5.42 mg/L to 1.08 mg/L for 2 days and 7 days of exposure, respectively, and for Gd from 3.67 mg/L to 1.38 mg/L. For all of the values obtained, Gd had a lower value, which indicates that in the conditions of exposure, it has a higher toxicity than La. Regarding the feeding rate, none of the La concentrations had a significant impact compared to the control ( $p > 0.5$ ). In contrast, exposure from 1 mg/L of Gd led to a significant decrease in the feeding compared to the control ( $p < 0.5$ ). In the somatic growth, both elements, significant inhibition of growth was observed for concentrations equal to or higher than 1 mg/L. Moreover, the lowest concentration of exposure (0.5 mg/L) of La induced a higher rate of feeding and a slight increase in size, in comparison to the control. As for the maturity, the results are expressed in terms of the EC<sub>10</sub>, corresponding to 0.45 and 0.42 mg/L for La and Gd, respectively, and the EC<sub>20</sub>, corresponding to 0.54 and 0.50 mg/L for La and Gd respectively. These results highlight the importance of focusing on sub-lethal endpoints for toxicity as the concentrations to cause mortality are higher and these endpoints can tell us more about potential negative effects on the population caused by more environmentally relevant concentrations.

#### 4.11.T-04 Rare Earths Increasingly in the Picture – But Where Do They Rank Among Other Metals Regarding Ecotoxicity and Occurrence in the Environment?

**Nele Deleebeeck, Andreas Fettweis, Laura Lefèvre and Michiel Claessens, Arcadis Belgium, Belgium**

Now that rare earths are increasingly being investigated, it is of utmost importance to compile all existing and newly generated information in order to update PNECs for use in environmental risk assessments. For instance, at the time of first submission of the REACH dossiers for various rare earth substances, little long-term or chronic ecotoxicity data were available for aquatic organisms, resulting in a high assessment factor for PNEC derivation and PNECs typically in the 0.1-1 µg/L range. In the meantime, long-term or chronic aquatic ecotoxicity data are increasingly becoming available and have learnt so far that such data do not necessarily bring a substantial improvement of the aquatic PNEC (< factor 5). At the same time, results from monitoring campaigns measuring rare earths in the environment are increasingly becoming available. Comparison of rare earth measurements in the aquatic environment with most recent PNEC values could give an idea of the potential for these elements to cause harm in actual water bodies. Even more important – keeping in mind the future requirement under REACH to demonstrate safe use taking into account potential mixture toxicity – is to get an idea of where the rare earths would rank among other metals when comparing their potential contribution to toxicity resulting from combined exposure. This could be done, as a screening approach, by calculating how much of the potential toxicity when assuming concentration addition would be explained by rare earths in environmental samples for which measurements of rare earths as well as other toxic or harmful metals are available. However, the unavailability of rare earth concentration measurements in large compilations of environmental monitoring data such as Waterbase hampers this exercise. Therefore, in this exercise, an attempt was made to link sampling locations for which rare earth concentration measurements are reported in recent scientific literature to sampling locations in Waterbase for which concentration measurements for other metals are available. This should be considered as a screening approach that gives a rough idea of where the rare earths could rank among the other metals. The results of this exercise could find application in the preparation for the mixed exposure assessment which will be imposed in the near future under REACH.

#### 4.11.P Progress in Knowledge of Rare Earth Elements

##### 4.11.P-Tu367 Rare Earths and Yttrium in tissues and shells of bivalves from the Danube River and its tributaries

**Keran Zhang<sup>1</sup>, Anna Lena Zocher<sup>2</sup> and Michael Bau<sup>2</sup>,** (1)CritMET – Critical Metals for Enabling Technologies, School of Science, Constructor University Bremen, Bremen, Germany, (2)CritMET – Critical Metals for Enabling Technologies, School of Science, Constructor University Bremen, Germany

Due to their numerous applications in various high-technology products and processes, Rare Earths and Yttrium (REY) have become microcontaminants in freshwater systems. Recent toxicological studies demonstrated that REY represent toxicities for aquatic organisms under laboratory conditions. Nevertheless, their biogeochemical behaviour, especially their uptake by aquatic organisms is still poorly understood.

Here, we report on the concentration and distribution of REY in the shells of four different mussel species, in the tissue and shells of three *Anodonta anatina* mussels, and in the respective ambient waters from the Danube River and some tributaries. The shells were grouped according to their sizes and then meticulously cleaned, while the soft tissues were dissected and lyophilized. All

samples were acid-digested and preconcentrated prior to ICP-MS measurements. Analytical quality was monitored by analysing the certified reference materials JLS-1 and BCR-668 for shells and tissues samples, respectively.

Size dependency of the REY concentration in shells was only observed for *Corbicula* shells, corroborating previous observations from the Rhine River. Shale-normalized REY patterns are similar for all shells and tissues, and they are slightly middle REY-enriched. Compared to the respective 0.2 µm-filtered ambient water, the biological samples show REY concentrations that are between three to five orders of magnitude higher, revealing significant bioaccumulation of REY. The observed preferential uptake of light REY relative to heavy REY is due to more stable complexation of HREY with dissolved ligands. Despite significant anthropogenic Gd enrichments in ambient water, there are no positive Gd anomalies in the biological samples, suggesting bioavailability of the Gd-based contrast agents present in the waters of the Danube River.

#### **4.11.P-Tu368 Toxicity of REE Along the Food Chain Microalgae-Daphnia in a 14-Day Nanocosm**

**Chantal K.E. van Drimmelen<sup>1</sup>**, Andrew Hursthouse<sup>2</sup> and Susanne Heise<sup>3</sup>, (1)Ecotoxicology, University of Applied Science Hamburg, Hamburg, Germany, (2)University of the West of Scotland, United Kingdom, (3)Hamburg University of Applied Sciences (HAW), Germany

While it is impossible in the lab to reflect the variability of responses and extent of interactions in natural systems to e.g. contaminant stressors, cosm-experiments can be set up that feature more than one species and run longer compared to single species bioassays. In order to find out, how rare earth elements (REE) impact the dynamics of organisms nanocosms were set up featuring two biological species from two trophic levels: the water flea *Daphnia magna* and its food organism, the microalgae *Raphidocelis subcapitata*. Our nanocosms consisted of PET-cups with characterized sediment (Lufa 2.2) and M4 media without EDTA. They were spiked with environmentally and anthropogenically relevant nominal concentrations of La or Gd (1, 10, 100 and 1000 µg L<sup>-1</sup>) and ran for 14 days. The experiment was repeated at least 5 times. Monitored endpoints comprised parameters such as death rate, reproduction rate, and population count, according to the guidelines of the single-species tests. Our hypothesis was that reproduction of *D. magna* would be a more sensitive endpoint after REE exposure compared to growth rate of *R. subcapitata*, and that the heavy REE gadolinium would be more toxic than lanthanum, in agreement with previous studies we had done. The nanocosms were analyzed over a course of 14 days using a sacrificial method. Preliminary results indicated that differences between effects under La and Gd exposure were not significant, probably due to the high variability in the nanocosms. In detail, reproduction of the daphnia was not significantly affected by the REE, meaning that this part of the hypothesis can be falsified. There was however, a clear trend for a delay of the onset of reproduction of daphnids between the control on one side, and La and Gd on the other. The growth rate of the algae also appeared to be impacted by REE. Analysis of data is being carried out and will be presented during the conference.

#### **4.11.P-Tu369 Toxicity of rare earth elements on human health**

**Antonios Apostolos Brouziotis**, University of Naples Federico II, Italy

Rare earth elements (REEs) are metals including the 15 lanthanides together with Yttrium and Scandium. China is the leading country in their exploitation and production. REEs are applied in several sectors like agriculture and medicine. Its application is necessary for the production of several technological devices, while they are used also as additives on diesel fuels. This extended use of REEs has raised concerns about their hazardness. This research belonging to PANORAMA project (grant n. 857989) focuses on the toxicity of REEs on both human and environmental health. We investigate the toxicity of REEs on three different levels: i) the human biomonitoring by analyzing the REE-content in human samples; ii) the ecotoxicity of REEs by elaborating ecotoxicity trials; iii) the environmental background of REEs by analyzing the REE-content in environmental samples. This presentation focuses on the procedure and results concerning the human biomonitoring. The occupational exposure of car mechanics to REEs was investigated. Urine and hair samples of workers were collected from two workshops, in Avellino and Lake of Como. REE levels were determined by ICP-MS. LOD and LOQ values for the instrument and matrices were determined before the sample analysis. The urines of Avellino workers contained significantly higher levels of REEs, creatinine and 1-hydroxypyrene, but REE levels in hair samples were not significantly different between the two groups. Two methods for preparing human urine samples were validated. According to a literature review of ours concerning the toxicity of REEs on human health, there are several ways of exposure to REEs and they have been associated with several diseases like anemia or endomyocardial fibrosis. REEs can be bioaccumulated in human body and cause several implications. More studies about their toxicity should be elaborated as their environmental concentration is increased overtime.

#### **4.11.P-Tu370 Does diffusion through soil control accumulation of REEs by plants roots or DGTs?**

**Luba Vasiluk<sup>1</sup>**, Bianca Pereira<sup>2</sup>, Amanda Pellegrino<sup>3</sup> and **Beverley A. Hale<sup>1</sup>**, (1)University of Guelph, Canada, (2)Golder Associates Inc., Canada, (3)MTE Consultants Inc., Canada

Despite the recent interest in assessing the risks associated with REEs, data on their bioaccessibility and bioavailability in soils are still relatively scarce. Diffusive gradients in thin films (DGT) are an *in situ* technique were used to measure Ce, Nd and Eu availability during prolonged deployments in an REE-amended black garden soil for comparison to accumulation of REEs by durum wheat (*Triticum durum* var. 'Kyle') and tomato (*Solanum lycopersicum* L.). The DGT attempts to mimic the accumulation of REEs by plant roots by providing a zero sink with high affinity for REEs. For all three REEs tested, a sigmoidal equation described the relationship between soil REE concentration and the rates of REE accumulation by DGTs, and of uptake by plant roots.

The results of these studies show that accumulation by DGT was faster and similar for Nd and Eu, but slower for Ce. It was demonstrated that durum wheat and tomato had comparable rates of accumulation for Ce and Nd and was about half of that estimated by DGT. Accumulation of Eu by durum wheat increased with soil REE concentration at about half the rate for tomato,

which was more comparable to DGT accumulation of Eu. Rates of accumulation by DGT and by plant correlate if diffusive limitation controls the plant accumulation. However, maximum accumulation rates for plants were up to 10-fold greater than those for DGT, for all REEs tested. The greater plant accumulations suggest that REEs' availability for plant root uptake does not only depend on diffusion of REE through the soil as measured by DGTs. Plants can also explore larger soil volumes for REEs uptake than DGTs which are subject to diffusion limitations. The results also suggest that durum wheat can take up Ce and Nd slightly more efficiently from soil even at lower REEs concentrations than tomato. The results suggest that even though Ce, Nd and Eu have similar ionic radii, they differ in affinity for plant roots, and thus the actual dose at a plant's biotic ligand does not depend only on diffusion of REEs through soils.

#### 4.11.P-Tu371 The Influence of Temperature Rise on the Impacts of Dysprosium to Adults and Sperms of the Species *Mytilus galloprovincialis*

**Carla Leite<sup>1</sup>, Tania Russo<sup>2</sup>, Alessia Cuccaro<sup>3</sup>, Gianluca Polese<sup>2</sup>, Amadeu M.V.M. Soares<sup>3</sup>, Carlo Pretti<sup>4</sup>, Eduarda Pereira<sup>5</sup> and Rosa Freitas<sup>3</sup>,** (1)Department of Biology, University of Aveiro, Aveiro, Portugal, (2)University of Naples Federico II, Italy, (3)Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal, (4)University of Pisa, Italy, (5)Department of Chemistry & LAQV-REQUIMTE, University of Aveiro, Aveiro, Portugal

The need for sustainable products has led to the increasing use of rare earth elements (REE) due to their unique properties. Dysprosium (Dy) is widely used in magnets, motors, electrical vehicles and nuclear reactors, being considered a critical element due to its economic importance and high supply risk. However, the increasing use of this element contributes to the enrichment of anthropogenic REE in aquatic systems. Nevertheless, the information on the potential toxicity of Dy is limited. Furthermore, nowadays, it is of utmost importance to consider climate change factors, such as ocean warming, since the risk of negative impacts derived from increased surface water temperature is predicted to be high to very high for bivalves. Moreover, the effects of pollutants can be amplified when combined with climate change factors. Thus, this study aimed to assess the effects of Dy (10 µg/L) in the species *Mytilus galloprovincialis* under actual (17 °C) and predicted warming conditions (21 °C). For this, histopathological and biochemical alterations were evaluated in adult mussels, as well as physiological alterations in sperm. A multivariate analysis showed three distinct groups, separating non-contaminated mussels at 17 °C, non-contaminated mussels at 21 °C and contaminated mussels at both temperatures. The first principal component (PCO1) separated on opposite sides mussels under stressful conditions from non-contaminated ones at the control temperature (17 °C). Dy-exposed mussels were strongly correlated with increased histopathological injuries and sperm motility while non-contaminated mussels under 21 °C showed a close association with higher lipid peroxidation levels and electron transport system activity. This study demonstrated that although Dy and increased temperature induced negative impacts on mussels, the combination of both stressors did not have an additive effect. Eventually, the tested stressors, specially Dy, at a long-term, may have consequences on mussels' reproduction capacity as well as their growth, abundance, and survival.

#### 4.11.P-Tu372 A biotic ligand model for acute toxicity of gadolinium on *Daphnia magna*

**Marion Revel<sup>1,2</sup>, Andrew Hursthouse<sup>2</sup> and Susanne Heise<sup>1</sup>,** (1)Hamburg University of Applied Sciences (HAW), Germany, (2)University of the West of Scotland, United Kingdom

The lanthanides (Ln) are a 15-element group that includes gadolinium (Gd). Because of the rising demand of Ln in contemporary technologies, they are now considered as emerging pollutant. Ln toxicity experiments persist to be challenging because of their complicated chemical speciation. The amount of free ion metal concentration in the water is decreased by the metal's complexation and precipitation with organic and inorganic substances. Free ion metals are most likely to interact with organisms by binding to ligands in membranes and causing physiological damage.

A biotic ligand model (BLM) takes metal speciation and interactive or competitive processes among metals for receptors into account in order to forecast the element's toxicity for a specific organism. This experimental setup's goal was to develop a first draft of a biotic ligand model for the acute toxicity of Gd for *Daphnia magna*. Acute daphnid tests lasting 48 hours were conducted in accordance with OECD 202 protocol to determine the stability constants of the cations and *D. magna* biotic ligand. Using a camera system, the *D. magna*'s mobility was assessed every 8 hours for 48 hours. In order to establish a link between cation concentration and the evolution of survival, only one cation's concentration was changed for each experiment. The combination of the Toxicokinetic-Toxicodynamic model (TK-TD) and the traditional BLM permitted the calibration of the model. Current results show a strong impact of Ca<sup>2+</sup> and Mg<sup>2+</sup> on Gd toxicity in contrast to that observed for K<sup>+</sup> and Na<sup>+</sup>. The stability constant calculations are currently in progress.

#### 4.11.P-Tu373 Toxicity and biodistribution of chronic dietary and waterborne of La and Gd in *Daphnia magna*

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The wide use of Rare earth elements (REE) in agriculture, medicine or new technology has led to an increase in aquatic systems. While the acute toxicity of these elements is increasingly known, there has been limited research on chronic toxicity, and none of these have looked at the impact of diet-borne metal toxicity. As a result, the goal of this work was to assess the toxicity and biodistribution of two REEs, lanthanum and gadolinium, on *D. magna* after chronic waterborne and food exposure. The waterfleas were exposed for 21-days to either La or Gd under four different conditions; (i) The control, in which *D. magna* was exposed to clean media and fed with non-contaminated algae (Ctr), (ii) Dietary exposure (D): Ln exposure through diet (contaminated algae) with *D. magna* grown in clean media, (iii) Waterborne exposure (W): Ln exposure through the medium (0.5 mg/L), and (iiii) combined exposure (DW): Ln exposure through both the contaminated microalgae as diet and the spiked media.

Every day, the organisms were fed, mortality and reproduction were checked. At the end of the 21 days of exposure, the daphnids were dried for 48 hours at 40°C. Dried samples were adhered with double-sided tape to the edge of glass slides in order to study metal biodistribution by X-ray fluorescence spectrometry (μXRF). After 21 days of exposure, no mortality was recorded regardless of the treatment. La combined exposure delayed the first brood by  $1.13 \pm 1.26$  days and Gd dietary and combined exposures led to a decrease of  $15.8 \pm 13.2$  and  $21.9 \pm 17.9$  neonates by living organisms respectively. Lanthanum seemed to uniformly accumulate in the gut regardless the condition of exposure. Gd accumulated in the hindgut after dietary exposure. Waterborne exposure permitted a more diffused biodistribution in the tissues of the organisms while the combined exposure led to a diffused distribution of the metal in tissues and in the intestinal tract. These findings highlight the relevance of dietary exposure for long-term toxicity.

**4.11.P-Tu374 Chaoborus punctipennis larvae to monitor rare earth elements contamination in lakes from two mining area**  
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Over the last decades, the mining exploration of Rare Earth Elements (REE) for high-technology industry has quickly increased, which leads to their diffusion and accumulation in aquatic environments as emergent contaminants. The use of sentinel species to assess REE contamination in such ecosystems is necessary for the development of ecological risk assessment tools. *Chaoborus punctipennis* larvae have been proposed as candidates to reflect the level of lake contamination of Cd, Se and Ni, but we do not know about their potential use for REE contamination. We aimed to evaluate the usefulness of these larvae to inform us of the REE distribution in lake water as well as to identify the environmental factors that can modulate the bioaccumulation of these contaminants. To do that, water and larval samples of *C. punctipennis* were collected between 2019 and 2021 from 15 lakes located in the Rouyn-Noranda (Quebec) and Sudbury (Ontario), two Canadian mining regions. The concentrations of organic matter, major cations, anions and metals including REE were measured in 0.45 μm - filtered water to estimate metal speciation. Also, REE concentrations in larvae were determined by ICP-MS/MS. Depuration experiments (72h) and adsorption essays were conducted in parallel of REE measurements. Our results show spatial heterogeneity of REE concentrations in the studied lakes with significant concentration gradients (maximum [REE]/minimum [REE] ratio) in water (La: 273; Ce: 232) and in whole-body bioaccumulation (ΣETR: 151; La: 654; Ce: 487). According to our results, depuration is not a necessary step in this species for all the REE. The percentages of REE measured as adsorbed on *C. punctipennis* varied from 47% (Ce) to 75% (Pr), being globally higher than those internalized. For the relationship between water concentration and bioaccumulation, significant correlations are observed between REE concentrations in water and those in larvae when REE free ion concentrations (and not total REE concentrations) are considered. These results show that chemical speciation plays an important role in the bioavailability of REE in lakes. Improved relationships are observed when different competing factors (e.g., Al, Fe) are included in such regressions. This information provides a better understanding of REE interaction with aquatic organisms, which is essential for monitoring studies of REE given their increased used in new technologies.

**4.12 Reducing Marine Pollution and the Role of Ocean Governance on the Road to Sustainability**

**4.12.T-01 Assessing Characteristics, Uses, and Potential Environmental Impact of Plastic Remediation Technologies**

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Plastic pollution is a global environmental issue. Remediation technologies to prevent or clean up plastic pollution are currently being deployed in households, on land, freshwater, and marine ecosystems. However, there is a critical need to classify, assess and regulate these technologies. In this study, we reviewed the scientific literature and web on plastic remediation technologies, created an overview of these devices, and investigated their characteristics (e.g., field of application, targeted plastic size). Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we conducted, on the electronic database Scopus, a systematic literature review on plastic remediation technologies. In addition, using similar search terms, we have performed a non-systematic Google search. From the systematic review, we observed a growing interest on plastic remediation technologies demonstrated by 34 out of 61 scientific studies published in the last three years. Of these 61 publications, 55 discussed plastic clean-up technologies. By merging the results of the systematic and non-systematic reviews, we created an overview of 124 plastic clean-up and prevention technologies with 29 of their key traits. Similarly to the result of the systematic review, the interest is currently on plastic clean-up technologies, which represent 93.5% of the technologies listed in our overview. Although clean-up technologies can have multiple fields of application, our results indicate that 60% of the 124 technologies, can be used in inland waterways, indicating that the attention is, to date, on the collection of plastic before it reaches the open ocean. Based on these results, we investigated the strengths, weaknesses, opportunities, and threats (SWOT) of this group of technologies. Our SWOT analysis indicates that regulations are needed when deploying a clean-up system, especially due to the environmental effect of these technologies on ecosystems. Despite the challenges, plastic clean-up technologies offer fundamental opportunities, from the more evident removal of plastic waste to raising awareness of plastic pollution, creating new job opportunities, and collecting data. Plastic remediation technologies are essential elements in the fight against plastic pollution. Therefore, their regulation is imperative to ensure a balance between the benefits and the potentially negative consequences.

**4.12.T-02 An Effect Assessment of Chemical Contaminants from the Salmonid Aquaculture Industry**

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Sea lice are naturally occurring parasitic copepods that feed on the mucous, blood and skin of both wild and farmed salmonid fish. In high numbers, they can reduce the general health and welfare of their hosts, cause significant economic losses to commercial salmonid farms and negatively affect wild salmonid populations. Consequently, the salmonid aquaculture industry relies on several Veterinary Medicinal Products (VMPs) to control sea lice infestations with treatments are administered topically or via medicated feed. Their discharge from open fish net pens and subsequent accumulation in different marine compartments, has raised significant concerns about their potential to negatively impact the marine environment.

The aim of this work was to comprehensively assess the sensitivity of aquatic species to six key anti-sea lice VMPs used in the Norwegian salmonid farming industry, including azamethiphos [AZA], deltamethrin [DELTA], hydrogen peroxide [H<sub>2</sub>O<sub>2</sub>], emamectin benzoate [EB], diflubenzuron [DIF] and teflubenzuron [TEF]. We carried out an extensive literature search in order to collate acute and chronic toxicity data for each of the selected VMPs. We subsequently compared the sensitivity of marine and freshwater species and finally estimated up-to-date predicted no effect concentration (PNEC) for both surface water and sediment compartments.

The findings of our literature search highlight that there has been extensive research into the acute toxic effects of many anti-sea lice VMPs. For example, we found 70, 299, 84 90 and 37 short-term reliable L(E)C<sub>50</sub> values reported in the literature for AZA, DELTA, H<sub>2</sub>O<sub>2</sub>, EB and DIF, respectively. These L(E)C<sub>50</sub> values represented 18, 64, 43, 30 and 14 different aquatic species, with crustaceans and insects generally the most commonly represented taxonomic group. There was no obvious difference in the overall sensitivity of marine and freshwater species, though differences in the taxonomic groups represented in each dataset may have biased these findings. Limited chronic toxicity data was available for all of the selected VMPs. Using the collated data, PNECs were estimated using the traditional deterministic method and the probabilistic method using species sensitivity distributions (SSDs). The estimated PNECs can be incorporated into future environmental risk assessments evaluating the risks anti-sea lice VMPs pose to the marine environment around aquaculture facilities.

#### 4.12.T-03 An Effect-based Toolbox for Petroleum Toxicity Testing Evaluated for Hazard Assessment

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After an oil spill, physical-chemical complexity of oil in addition to unique spill site-specific factors result in a variety of hazard scenarios for the exposed biota. Information about the oil toxicity is needed, which can often not be derived from quantified individual oil constituents due to unknown compounds and mixture effects. Effect-based methods (EBMs) can complement chemical analysis to assess the toxicity and hazard and predict future consequences for oil-exposed biota. EBMs can even be used for post-spill biological monitoring. Methods combining a huge set of biological effect data for a comprehensive toxicity evaluation often include the transformation of data by normalization to a maximum induction or reference site, which reflects the dimension of the biological relevance only marginally.

Hence, the aim of the present study was to evaluate a set of different EBMs for three petroleum products of varying processing degree in one common context. Toxicity assays of one crude oil and two refined petroleum products were performed with and without the addition of a chemical dispersant. Biological effect data of 12 different species/cell lines with a range of endpoints were included. Small scale *in vitro*-based methods (e.g. receptor activation) as well as methods using whole organisms of a broad range of developmental stages (from early embryo to adults) and salinity regimes as well as a set of biomarkers (e.g. enzyme activity) were included in the analysis leading to, e.g., a set of 41 endpoints for the crude oil. Data from all biological endpoints were individually transformed into effect classes between 1 and 5 ranging from no (1) up to high effect (5). The classification system combined mathematical approaches and expert judgement to account for the dimension of biological response and relevance. Weighting factors were introduced to distinguish between well-established and rather uncertain endpoints. Multivariate Principal Component Analysis (PCA) was performed with classified effect data.

Based on the PCA, a petroleum-specific bioassay battery providing EBMs being sensitive endpoints for the hazard assessment of oil contamination is suggested. A special advantage of the current study is the use of identical oil batches and shared sample preparation protocols allowing a large conformity and hence a joint evaluation. The suggested bioassay battery can be applied for monitoring programs and for the hazard assessment of e.g. new fuel types.

#### 4.12.T-04 Ecotoxicological Effects of Marine Scrubbers' Discharge on Multi-domain Microbial Communities At Molecular Biodiversity Test Endpoints

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Exhaust gas cleaning systems (scrubbers) on-board vessels discharge effluent water with high concentrations of potentially toxic substances, including Polycyclic Aromatic Hydrocarbons (PAHs) and metals, and have acidifying and eutrophying properties that

synergistically can affect biota and ecosystem health. The increased shipping traffic in Eastern Mediterranean, especially near busy port areas, may increase pressures caused by scrubber discharges in the seawater. Microbial communities in marine systems are the first responders to exogenic stresses, such as pollutant surges, and certain members can absorb the pulses via biodegradation pathways, or others can magnify the outcomes via bioaccumulation to higher trophic levels. We performed mesocosm experiments to examine the responses of the multi-domain marine microbial communities of two coastal areas in Eastern Mediterranean, with variable trophic states (polluted and unpolluted), to different scrubber influxes. High-throughput metabarcoding targeted the bacterioplankton and unicellular eukaryotic assemblages, including phytoplankton and protozooplankton, to reveal community structural shifts; and random metagenomic sequencing aimed to provide insights into the functional strategies employed by microbes towards stressor alleviation. The results point towards variable community responses of the prokaryotic and microeukaryotic water-partitions, with shifts in biodiversity and composition. Among bacteria, a proliferation of Operational Taxonomic Units (OTUs) closely related to PAHs degraders was apparent in both low (1 % v/v scrubber concentrations) and high (10 % v/v) scrubber inputs. This was coupled with the presence of pathways connected to the functional groups of environmental information processing and aromatic compound metabolism.

#### **4.12.T-05 Assessing Cumulative Risk of Metals and Polycyclic Aromatic Hydrocarbons from Ship Activities in Ports**

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Studies show that the shipping sector accounts for a variety of different contaminants, e.g. metals and polycyclic aromatic hydrocarbons (PAHs), are released to the marine environment. Metals and PAHs are found in several contaminant sources from ships, e.g., atmospheric deposition from exhaust; release of biocides from antifouling paints and discharge from onboard operations such as bilge water from the engine room and discharge of scrubber water, i.e. water from exhaust gas cleaning systems. Today, the discharge of scrubber water is only regulated by guidelines of recommendatory nature, despite ecotoxicological experiments showing adverse effects on marine organisms. Earlier this year, the Marine Environment Protection Committee (MEPC) of the International Maritime Organization (IMO) proposed new guidelines on how to assess risk and impact from scrubber water discharge, to aid member states when formulating local regulations. The aim of this work was to examine the applicability of the new guidelines.

Also, the need to adopt a more complete approach was identified, where the contribution from several contaminants and contaminant sources are included when assessing the environmental risks associated to scrubbers and shipping in general. Therefore, an additional aim was to compare the metal and PAH load from different contaminant sources and to assess their contribution to cumulative risk in the marine environment. Discharge data from the ship-activity model STEAM, together with previously derived emission factors, were used as input to the hydrodynamic and chemical fate model MAMPEC, to calculate predicted environmental concentrations (PECs) of PAHs and metals in four different ports. The calculated PECs were used to assess the environmental risk by comparing the PECs with predicted no effect concentrations (PNECs), yielding risk characterization ratios (RCRs) for each contaminant. The loads and the associated risks from different sources were assessed both separately and combined by summarizing the RCRs from metals and PAHs. The results show that the proposed guidelines will not provide adequate protection of the marine environment and that scrubbers and antifouling paint contribute most to the contaminant load and risk in ports, where three out of four ports exceeded the limit for unacceptable risk. This study highlights the importance of accounting for multiple stressors and their cumulative effects when evaluating the environmental risks of shipping.

#### **4.12.P Reducing Marine Pollution and the Role of Ocean Governance on the Road to Sustainability**

##### **4.12.P-Tu375 Ecotoxicological Effects of Three Different Scrubber-Waters on The Larval Development of The Polychaeta *Sabellaria alveolata***

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In response to the sulphur regulation rules approved and adopted by the International Maritime Organization, many shipping companies have invested in scrubber-water systems instead of using fuels with a lower sulphur percentage, transferring atmospheric pollutants (SO<sub>x</sub>, NO<sub>x</sub> and CO<sub>x</sub>, PAHs, metals and particles) to the seawater. This decision was made without any risk assessment on scrubber-water effects in marine and coastal ecosystems. So, the present work was focused on the ecotoxicological effects of scrubber-waters on the larval development of seawater organisms.

The larval development bioassays were carried out with the marine polychaete, *Sabellaria alveolata*. Since this polychaete releases the gametes into the water, the embryonic development was affected by the seawater quality. In this bioassay, three types of scrubber-water produced with Atlantic seawater were used: scrubber-water A (SWA) artificially produced and Scrubber-water B and C (SWB, SWC) produced on board two different ships. The scrubber-waters were tested at distinct concentrations: control; 0,001; 0,01; 0,1; 1,0; 10,0; 25,0; 50,0 and 100,0%, either with or without pH correction.

The scrubber-waters were analysed, showing very low pH values and an enrichment of PAHs and alkylated-PAHs, as well as trace metals. By comparing the concentration of PAHs and alkylated-PAHs, the magnitude order found for scrubber-waters was: SWC > SWB > SWA. Concerning the trace metal levels, they varied according to the elements. V, Zn, As, Cd and U were found higher at SWB while Cr, Mn, Fe, Co, Ni, Cu, Hg and Pb at SWA. SWC was not analysed for metals because the sample was lost. The exposure of *S. alveolata* embryos to scrubber-waters evidenced concentration-dependent effects on the larval development abnormality. Effects on larval stages were similar to the three scrubber-waters without pH correction and occurred at

concentrations sensibly lower, with NOEC  $\leq 0,001\%$  and LOEC = 0,01%. The scrubber-water more toxic was the SWA presenting an EC<sub>50</sub> of about 4%. While the SWB and SWC were less toxic, presenting an EC<sub>50</sub> of 9 and 10% respectively. All the assays performed with pH correction, showed lower toxicity, which can be explained by the decrease in metal's bioavailability. The current results showed that scrubber-water are highly toxic to the tested species. Furthermore, the original polychaeta larval development bioassay showed to be a suitable to assess the risk of scrubber-waters on marine aquatic species.

#### **4.12.P-Tu376 Chronic Toxicity Evaluation Using Sea Urchins of Chemical Additives Present in Oilfield-Produced Water**

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The largest volume of effluent from oil and gas exploration and production is oilfield-produced water. In evaluating how produced water behaves in the ocean, it is necessary to know the fate of the components once in the environment and their biological effects, including acute and chronic toxicity, at the concentrations and time of exposure in the environment.

Chemicals are added to the oil or gas field to manage operational problems, such as assisting in the recovery and pumping of hydrocarbons, preventing corrosion in the system, facilitating the separation process of oil, gas and water, and preventing methane hydrate formation in gas production systems. Among the chemical additives are biocides, surfactants, antifouling agents, demulsifiers, defoamers, and others. Part of the chemical additives is disposed of into the sea along with the produced water because they are water-soluble.

This work evaluated the short-term chronic toxicity with sea urchin embryos (*Echinometra lucunter*) with chemical additives present in oilfield-produced water: antifouling, defoamer, surfactant, biocide, reducing agent and demulsifier in the commonly used dosages. The tests were performed according to the procedures described in Brazilian Standard.

The results indicated that most of the chemical additives at the concentrations evaluated represent a high risk to aquatic organisms, with the biocide and demulsifier showing the highest chronic toxicity, even at low concentrations. The NOEC (no observed effect concentration) values obtained were defoamer, 10 mg/L; antifouling agent, 2.5 mg/L; biocide, 0.78 mg/L; demulsifier, 0.35 mg/L and surfactant, 75 mg/L. The reducing agent did not present toxicity at the concentration evaluated (20 mg/L).

This work highlights the importance of evaluating the harmful effects of chemical additives commonly used in the oil platform and the need for treatment processes to reduce/remove these toxic compounds before they cause damage to aquatic biota.

#### **4.12.P-Tu377 Oil in the Sea: Inputs, Fates, and Effects**

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Oil and natural gas represent more than 50 percent of the worldwide energy supply, with high energy demand driven by population growth and improving standards of living. As the consumption, exploration, transportation, and production of oil has increased to meet demand, so have studies of the fate and impacts of oil in the sea. Those studies have helped regulators implement new safety requirements, spill responders to develop innovative techniques, and industry to employ updated operational practices and safety measures to limit the impact of oil on the marine environment. This presentation summarizes the 2022 U.S. National Academies of Sciences, Engineering and Medicine's consensus study, Oil in the Sea IV: Inputs, Fates, and Effects. The report includes major findings on the current state of knowledge on, and the committee's recommendations for, reducing inputs of oil into the seas, as well as for improving understanding of the fates and effects of oil in the marine environment and for reducing the more harmful environmental effects. The study is the forth in a series of Oil in the Sea reports. Since the last study was released, almost 20 years ago, there have been tremendous advances in knowledge concerning how oil in marine waters behaves and effects organisms. These advances are based on improved, and in some cases, new technologies and voluminous numbers of new studies performed by government agencies, industry, and academia. One of the main drivers for this enormous growth in knowledge was the research funds provided after the Deepwater Horizon incident. The National Academies was empaneled to review the current state of knowledge on oil in the sea with specific tasks that included: compiling an inventory of the sources, composition, and quantity of hydrocarbon inputs to the marine environment, as well as mitigation measures, with an emphasis on North American waters; including an assessment of the state of the science on the fate and effects of fossil fuel hydrocarbons in the marine environment.

#### **4.12.P-Tu378 Assessing the Acute Toxicity of Herders to *Skeletonema* sp and *Tisbe battagliai* in Accordance with the UK Procedures for the Testing and Approval of Oil Spill Treatment Products**

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A herding agent (or herder) is a chemical product containing a surface-active ingredient which reduces the surface tension of water to change how an insoluble liquid like crude oil spreads, causing oil slicks to rapidly thicken, making them easier to burn. Herders are commercially ready for use; however, they require appropriate regulatory approvals before they can be considered for use in event of an oil spill. As such, the acute toxicity of a herding agent to the marine alga *Skeletonema* sp and marine copepod *Tisbe battagliai* was determined in accordance with the UK procedures for the testing and approval of Oil Spill Treatment Products (CEFAS, 2020) to allow submission of the product to the MMO (Marine Management Organisation).

The determination of acute toxicity to *Skeletonema* sp generated a fluorescence intensity (measured as a surrogate for algal biomass) EC<sub>50</sub> of 25.2 mg/L and a growth rate EC<sub>50</sub> of >100 mg/L. The determination of acute toxicity to *Tisbe battagliai* generated an LC<sub>50</sub> of 447 mg/L.

Based on assessments of products currently on the UK approved products list, the pass/fail criteria for the testing and approval of Oil Spill Treatment Products sets a threshold EC<sub>50</sub> limit of >10 mg/L. As the product exceeded this limit in both tests discussed, the criteria were passed, and the product would be suitable for addition to the UK approved products list.

#### **4.12.P-Tu379 Relationships Between Tissue Polycyclic Aromatic Hydrocarbon (PAH) Profiles and Biomarker Responses in Baltic Sea Mussels: A Study Using Compiled Laboratory and Field Exposure Data**

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Polycyclic aromatic hydrocarbons (PAH) remain a ubiquitous component of marine pollution. Apart from exposures related to oil spills and other extraordinary releases of these substances, their presence in the marine ecosystem is relatively constant due to atmospheric deposition. Their role in total toxicity of anthropogenically impacted marine waters remains often uncertain although different PAH exert a wide range of effects from acute toxicity to carcinogenesis and endocrine disruption. For this study, extensive chemical-biological data sets collected from previous laboratory oil exposure experiments and field caging studies at sites characterised by different degrees of pollution using Baltic mussels (*Mytilus trossulus*) in the northern Baltic Sea were compiled. Multivariate statistical analyses were carried out examining the relationships between selected biomarkers measured in individual mussels and tissue concentrations of different PAH measured in pooled samples from the same experimental treatment or field site. The biomarkers included consisted of biochemical parameters associated with the antioxidant defence system (glutathione *S*-transferase, glutathione reductase, catalase and superoxide dismutase activities, and lipid peroxidation), neurotoxicity, (acetylcholinesterase activity), genotoxicity (micronuclei and other chromosomal aberrations), and general health at the cellular level (lysosomal membrane stability). In addition, data on other contaminants measured in the tissues of mussels (organochlorines, organotin and trace metals) as well as relevant abiotic environmental factors were included in the analyses as explaining parameters. The results indicate that certain patterns of biomarker responses are associated with the PAH profiles observed in the tissues (e.g., abundance of low or high molecule weight PAH, methylated PAHs, benzo compounds). Thus, the expected sublethal impacts are dependent on the type of PAH contamination and the profile of bioavailable compounds in the environment. The results of the analysis can be used for an improved risk assessment of marine sites characterised by different types of PAH contamination.

#### **4.12.P-Tu380 Dumped Chemical Warfare Agents - Acute Toxicity and Sublethal Effects in Aquatic Organisms**

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After the World War II, massive quantities of chemical warfare agents (CWAs) were dumped into the seas worldwide. Only in the Baltic Sea approximately 50,000 tons of chemical weapons were disposed of while in the adjacent Skagerrak Strait the estimated amount reaches 170,000 tons. By now, many of the shells and casings of munitions have corroded to the extent that the CWAs are leaking to the water and sediments. The possible impact of dumped CWAs on aquatic ecosystems is scarcely studied, and especially the degradation products of different CWAs and their toxicity to aquatic organisms are largely unknown.

In the present study, the phenylarsenic agents Clark I (DA), Adamsite (DM) and triphenylarsine (TPA) and their degradation products, such as DA related diphenylthioarsinic acid (DPTAA), were identified in the sediments collected from the Bornholm Basin dumping site. Acute toxicity of these compounds was tested using water flea *Daphnia magna* (48 h immobility test) and zebrafish *Danio rerio* (fish embryo acute toxicity test until 120 hours of post fertilization, hpf). After establishing their acute toxicity, very low effect concentrations ( $\leq$  EC<sub>10</sub>) of some of these compounds were further tested to detect possible sublethal effects applying biomarkers of oxidative stress, biotransformation activity, neurotoxicity and behavioral responses.

In *D. rerio* the EC<sub>50</sub><sub>120hpf</sub> was 80-250 µg/L for DA, DPTAA and DM, no effects were observed for TPA and TPA-related compounds. *D. magna* were even more sensitive and showed acute effects for DPTAA, DA, DM and TPA; the EC<sub>50</sub><sub>48h</sub> values were 32, 50, 250 and 417 µg/l, respectively. TPA-O did not induce immobility and TPA-S had partial immobility at 400 – 9000 µg/l. Significant biomarker effects were also observed in both test species exposed to sublethal concentrations compared to the control treatments. Similar or even higher toxicity effects are expected for methylated degradation products of DA for which the analyses are currently ongoing.

These results show that CWA compounds and their degradation products present in the sediments possess a high toxicity potential for aquatic species. Since environmental conditions and human activities may change in the dumping areas, it would be important to monitor the concentrations of the CWA related chemicals including their toxic degradation products as well as possible biological effects related to the CWA contaminated sediments.

#### **4.13.P Scientific Advances in Understanding Environmental Fate and Effects of Polymers**

##### **4.13.P-Th330 A Proposal For a Three-tiered Approach for Standard Information Requirements for Polymers Requiring Registration Under REACH**

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Polymers are a diverse set of chemistries, part of our daily life and provide a multitude of technical functionalities. As part of the EU Commission's Chemical Strategy for Sustainability (CSS), there are efforts to develop environmental and human health standard information requirements (SIRs) for man-made polymers requiring registration (PRR) under a revised Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation.

By their nature, polymers are different to small and discrete molecules and conventional risk assessment approaches cannot be applied to most polymers. Therefore, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Polymers Task Force has developed a conceptual three-tiered proposal to generate data to assess individual and groups of polymers requiring registration (PRR). A prerequisite is the grouping of polymers according to three criteria: chemistry, physico-chemical properties and hazard similarity. The proposed approach considers the limited bioavailability of polymers as a prominent difference to many small molecules and takes this into account as a central element, in particular for human health. Among others, methods for assessing a potential for systemic bioavailability are integral to Tier 1. Within Tier 1, basic data will be generated on a single polymer or polymer group making use of non-vertebrate testing methodologies. Decisions for further studies at higher tiers are based on considerations of properties and effects, combined with systemic bioavailability, and use and exposure considerations. For many PRRs, Tier 1 data on hazard, use and exposure will likely be sufficient for achieving the protection goals of REACH. Vertebrate animal studies and higher-tier fate testing in Tiers 2 and 3 can be focussed on testing needs according to the combined factors above.

The ECETOC Polymers Task Force is of the opinion that the principle of a tiered approach with no vertebrate animal testing at Tier 1, followed by justified, limited and targeted vertebrate testing in higher tiers considering systemic bioavailability estimates, use and exposure considerations and material properties first, provides a basis for modern and intelligent data generation for the next decade as well as an adequate level of protection of humans and the environment.

#### **4.13.P-Th331 An Integrated Approach to Assess the Chronic Effects Induced By Water-soluble Polymers**

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Water-soluble polymers (WSPs) represent a new class of emerging contaminants whose widespread use in several applications such as detergents, paints, pharmaceuticals, personal care products or pesticides determined their release into the environment. Despite this large use, their environmental presence and concentration are unknown and their behaviour and impact in the ecosystem is unclear. This could be attributed to the fact that this WSPs do not fit into the polymer size-based classification, escaping the current legislations towards physical contaminants as plastic.

For this reason, the aim of the present study was the evaluation of toxicity induced by three of the most common used WSPs: the polyvinylpyrrolidone (PVP), polyacrylamide (PAA) and polyethylene glycol (PEG). We assessed the chronic toxicity of different WSP concentrations (1 µg/L, 0.5 mg/L and 1 mg/L) in the fish *D. rerio* (exposure from 0 to 120 hours post fertilization - hpf).

For the assessment of the chronic toxicity and to understand the mode of action of these WSPs, we evaluated the effects at a high level of biological organization through the monitoring of the alteration on the swimming performance of the exposed specimens. Besides, the high-throughput technology of proteomics has been assessed on the organisms exposed to the highest concentration (1mg/L) of all the three WSPs to investigate the effects at deeper level of the biological complexity and to investigate the possible mechanism of action of these WSPs.

The results regarding *D. rerio* locomotor response to different cycles of light/darkness, underlined PVP as the most effective WSP, since it affected behavioral parameters (total distance moved, mean velocity, mean mobility) at all the concentrations. The same parameter has been affected after the exposure to the highest concentrations of PEG, while PAA seemed the less effective. Concerning proteomics results, PEG and PVP showed a similar way of action impacting on proteins mainly involved in genetic processes, while PAA showed a different pathway of action, impacting most on proteins involved in protein binding and transport.

#### **4.13.P-Th332 Bridging the Gap – An Analytical View on Data Requirements for Polymer Risk Assessment**

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Synthetic polymers are integral enablers of modern life, due to their versatility and ease of production. Their usefulness renders them ubiquitous in the modern world leading to an increasing build-up in different terrestrial and aquatic ecosystems. This ongoing development is generating considerable concern from the public, leading to requests to close the perceived data gaps on identities, properties, use patterns and fate of polymers in the environment.

To close these knowledge gaps the European Chemicals Strategy for Sustainability extends the substance registration under REACH to all polymers on the European Market. The registration process will be based on a stepwise approach, comparable to the registration of small molecules. In the initial phase all polymers sold on the European market need to be notified and the polymers requiring registration (PRRs) have to be identified using a set of to be defined criteria. These criteria should be science-based and together with a clear guidance on data generation facilitate the registration of this heterogeneous substance class. As PRR decision, grouping, downstream (eco)toxicological studies and subsequent risk assessments rely on the availability of high-quality data, robust methods and clear guidelines for the characterization of polymers are needed.

The sheer number of polymers on the European market results in an urgent need for standardized analytical methods to facilitate polymer data generation and risk assessment. In contrast to small, defined substances polymers are by definition mixtures, thus complicating sample preparation, measurements and data interpretation. Defining robust data requirements is no trivial task as existing test methods (e.g. OECD or ISO methods) are not necessarily transferable to polymers and can result in unreproducible or misinterpreted data and biased risk assessments if not modified for use on polymers. In case of other endpoints adapted from small molecule REACH, suitable and validated test methods do not exist and need to be developed.

This presentation will focus on exemplary requirements (e.g. molecular weight, solubility, log Pow) relevant for PRR classification and risk assessment to illustrate the analytical challenges imposed by this complex substance class and the potential influence on subsequent assays and risk assessments. Practical examples based on the currently available methods underline the need for new methods and clear guidance on the testing of polymers.

#### 4.13.P-Th333 Influence of the PH Value to the Degradation of Ester-Based Thermoplastic Polyurethanes

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Microplastics are solid polymeric particles with a size of 1-1000 µm (ISO/TR 21960:2020), which can be emitted from mismanaged waste into the environment, where microplastic is now ubiquitous. What happens to the microplastics after ending up in the environment, which risks entail and what effects it has are not sufficiently clarified up to now. The most certain issue is that the plastic particles in the environment are exposed to natural ageing, are fragmenting and degrading, such that the potential risk to ecosystems and humans is increasing due to the formation of smaller and smaller particles, potentially even including nanoplastics, if these are ingested before their further degradation. Therefore, and in view of a possible registration of polymers under REACH in the future, it is necessary to investigate the degradation of thermoplastic polyurethanes (TPU) regarding hydrolysis stability to evaluate possible risks and effects to the environment.

In the present studies, one thermoplastic polyurethane – with and without hydrolysis stabilizer – is exposed to different pH buffers at 50°C for 14 days to investigate hydrolysis depending to different pH values (acid, alkali and neutral) based on OECD guideline TG111. The hydrolysis behavior of the TPUs is characterized by surface sensitive techniques and on bulk properties. First degradation effects can be detected by SEC. Hydrolysis, especially under acidic and basic conditions, leads to chain scissions to lower molecular masses. Furthermore, the degradation products which indicate the structure of the bulk material were detected by thermo-analytical methods like TGA-FTIR for the small degradation products and the thermo extraction/desorption-gas chromatography/mass spectrometry (TED-GC/MS) for bigger degradation products. Acidic and basic hydrolysis shows the same degradation behavior which is caused by a preferred scission of the ester and urethane functionalities. Surface-sensitive techniques such as XPS demonstrate less carboxylic acid formation at acidic than at alkaline pH value in the TPU without stabilizer, whereas the TPU with stabilizer ages to the same extent in both pH ranges. Altogether, the hydrolysis of TPUs – independently of added stabilizer or not – in acid and alkali environment is accelerated compared to the neutral hydrolysis.

#### 4.13.P-Th334 Regulatory Developments in Environmental Hazard Assessment of Polymers Under REACH

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Historically, regulatory frameworks have considered polymers of lower hazard than non-polymers, as a result of their high molecular weight (MW). The assumption is that high MW is associated with poor bioavailability and hence low toxicity. This is supported by the ‘rule of five’ (Ro5) which correlates certain molecular parameters and descriptors (molecular weight, logarithm of the octanol-water partition coefficient - logKow, number of hydrogen bond donors - HBD, number of hydrogen bond acceptors - HBA) with the likelihood of a substance to cross biological membranes. In particular, the Ro5 posits that substances with a MW > 500 Da have poor absorption and permeation, thus their (systemic) bioavailability will be limited. However, 20 years from the introduction of the Ro5, scientific research demonstrates that the 500 Da cut-off is questionable. This is illustrated by a substantial increase in the amount of oral drugs with MW > 500 Da (and therefore capable to be systemically available) approved by Food and Drug Administration (FDA) since 2000 [1]. Pharmaceutical literature reports that molecules with MW > 800 Da (e.g., paclitaxel) or even > 1200 Da (e.g., cyclosporine) are not hindered in their membrane permeability [2]. With this change in paradigm, high molecular weight substances such as polymers can no longer be regarded as innocuous by default.

The objective of this presentation is to highlight the areas where more research is needed to understand the polymer’s bioavailability and in support of the upcoming development in hazard assessment of polymers under REACH. In this context, a tiered approach is being proposed which includes reduced information requirements for certain types of polymers based on assumptions that their large molecular size limits transport across membranes and therefore their bioavailability.

#### 4.13.P-Th335 Analytical Approaches to Characterize and Quantify Process Related Minor Constituents/Impurities in Polymers

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In the context of REACH the view on polymer assessment is in transition and a more thorough characterization will be needed. Besides the molecular weight distribution and content of monomeric substances, there are further constituents/impurities which could originate from the manufacturing process. Oligomeric reaction products, residual solvents and catalysts could be targeted. Our aim is to discuss the capabilities and limitations of conventional chromatographic techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC) using different detectors and mass spectrometry.

Identification of such process related minor constituents could be straight forward in many cases while quantification is difficult to achieve due to the lack of readily available reference substances. Thus, alternative quantitation approaches will need to be developed and assessed. While GC-FID (Flame Ionization Detector) provides a carbon based response and is a well-established tool for standard-less quantification, responses in HPLC detectors typically vary dependent e.g. on the molecular structure.

#### 4.13.P-Th336 Applicability of Standard Environmental Fate and Effects Methodologies for Non-ionic Water Soluble Polymers

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There has been an increasing regulatory interest in the environmental hazard and fate profile of polymers. Discussions to include polymers under REACH regulation are on-going. Regulatory submissions require a suite of standard assays to characterize a compound's hazard and risk. It is therefore important to assess and validate these existing methodologies and any modifications needed when evaluating polymers. In this study, we focused on evaluating water soluble polymers commonly used in the down the drain products including polyethylene glycol, polyvinyl alcohol, and carboxymethyl cellulose. Polymers evaluated had varying structural characteristics. We applied standard environmental effects assays including OECD 201 algal growth inhibition assay, OECD 202 acute Daphnia immobilization assay, and OECD 236 fish embryo acute toxicity (FET) assay to evaluate applicability. Further we applied standard environmental fate methods for down the drain chemicals to assess method applicability and modifications needed when evaluating biodegradation of polymers. These methods included the OECD 301B Ready Biodegradability TG and the OECD 302B Zahn Wellens TG. In addition, we assessed the presence of competent degradation in river water using non-standard assessment methods. Results will be discussed and recommendations regarding method modifications will be provided.

#### **4.13.P-Th337 Aromatic Isocyanate-based Polymeric Prepolymers: Investigation of Structure-Property Relationships to Aquatic Exposure and Acute Aquatic Toxicity**

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The future registration of polymers in the EU will require the submission of hazard data. A testing program has been performed to explore the potential for exposure and associated health and environmental hazards of aromatic isocyanate-based polymeric prepolymers. The precursor Diisocyanate (DII) substances can be of concern during manufacture or application of polyurethane (PU) raw materials. The hazard potentials can be reduced by the reaction of DII precursors with various polymeric polyether and polyester polyols, which meet the OECD definition of a polymer, to produce isocyanate-terminated prepolymers. This work explored how variation in structures of the DII precursors and polyol building blocks and in the physical-chemical properties of NCO-terminated prepolymers, such as log  $K_{ow}$  and molecular weight (MW), contributes to their aquatic exposure and hazard potentials with residual DII precursor content < 0.1%. A group of 10 aromatic prepolymers ranging in MW from 750 – 6,600 g/mol, NCO-functionality from 2 – 4, and calculated log  $K_{ow}$  from -5 to +45 were synthesized from among different DII precursors and polyol types. The aquatic exposure potentials were determined using OECD Guideline 120, from which the water-accommodated fractions (WAF) were obtained for testing acute immobilization of *Daphnia magna* according to OECD Guideline 202. For prepolymer loading rates of both 100 and 1000 mg/L in water and prepolymers with a calculated log  $K_{ow}$  value of > 10, water-extractable reaction products (measured as dissolved organic carbon, DOC) were not quantifiable. Below log  $K_{ow}$  of 10, the quantifiable DOC concentrations showed an expected inverse correlation with calculated log  $K_{ow}$ .

All prepolymer substances independent of log  $K_{ow}$  exhibited 48-h median effective loading rates (EL<sub>50</sub>) of > 100 mg/L. Thus, the acute aquatic hazard potential for this NCO-terminated prepolymer substance class is very low, regardless of structure features, physical-chemical properties, or magnitude and composition of their WAF. The revelation of these trends in aquatic exposure and hazard potentials could now provide a basis for regulatory screening of aromatic-NCO-based polymeric prepolymers, while also informing the design of such substances having reduced exposure and/or hazard profiles.

#### **4.13.P-Th338 Assessing the Potential Role of Bioinformatics Tools in Future Polymer Biodegradation Testing**

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In recent years, our knowledge of molecular Biology and Bioinformatics has undergone a massive expansion, and this has paved the way for the development of *in silico* tools that can predict biodegradation of a test compound. However, these tools were developed for low molecular weight chemicals and have not been assessed for their applicability in predicting polymer biodegradation. If effective, such tools could form part of a new framework for assessing the biodegradability of polymers, providing an insight into this that may inform the choice of subsequent lab tests carried out on the polymer. To fill this knowledge gap, we assessed two of the most prominent biodegradation prediction tools currently available: enviPath and PathPred by testing them on a range of monomers and oligomers that form larger molecular weight polymers. We compared their predictions for the biodegradation of these polymers, with and without various modifications. There was significant disagreement between the tools on the biodegradation of many polymers' constituent oligomers, suggesting that one or both still need refinement before they can be considered reliable. Agreement on the presence or absence of biodegradation pathways was found only 47% of the time. Therefore, the tools are likely not yet ready for integration into polymer biodegradation testing frameworks but do show promise, with further refinement tools like these could be a useful addition. It would be beneficial for the tools' predictions to be verified with lab-based biodegradation tests on the chosen polymers and oligomers in the future. The results of these tests could indicate which of these tools, is more accurate and therefore has greater potential for improvement that may make it effective to a future polymer biodegradability assessment framework.

#### **4.13.P-Th339 Challenges and Limitations in the Determination of Molecular Weight and Oligomer Content of Polymers with Regard to Risk Assessment**

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Polymers are large molecules, that are composed of similar or identical repeating units, the monomers. Due to the nature of polymer synthesis, polymer materials show distributions instead of a single value in many properties, of which molecular weight is probably the most important one. The molecular weight of the polymer molecules has a strong influence on their solubility,

mobility and therefore availability to the environment. Consequently, the molecular weight distribution (MWD) and values derived from it – such as the oligomer content – is an important input for the risk assessment of polymers.

There are several methods to determine an average molecular weight of polymers, but only fractionation techniques can yield the full MWD. The state of the art technique is Size Exclusion Chromatography (SEC or GPC) and two OECD guidelines (118 and 119) are devoted to the application of SEC in polymer characterization. Currently there are no alternatives to SEC for the determination of the oligomer content, also given as amounts < 1000 g/mol and < 500 g/mol.

However, SEC is not universally applicable to all polymer products. Not all polymers are soluble, which is a prerequisite for SEC. Some polymers may be crosslinked or of ultra-high molecular weight, which exceeds the separation limit of SEC. For certain polymers, a large amount of salt or modifier needs to be added to the solvent, which often interferes with the oligomer content determination. Sometimes, no suitable column is commercially available. SEC, as a relative method, depends on suitable calibration standards. The availability of those can also be challenging.

In summary, despite SEC being a well-established technique, there are many pitfalls and exceptions to consider when one determines the MWD and the oligomer content of polymers. For some of those limitations there are approaches on how to resolve them. However, determination of MWD and oligomer content is not possible for all polymers.

This contribution will provide an overview on the current limitations of the application of SEC to characterize polymers, which is important for their risk assessment, and present strategies to overcome or circumvent some of these challenges.

#### **4.13.P-Th340 Development of a Testing and Evaluation Methodology for Polymeric Substances in Soils**

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Agricultural management faces new challenges due to climate change and simultaneous population increase. The optimum crop emergence is therefore a high priority objective. By developing new innovative multifunctional seed coating systems, the Fraunhofer PREPARE project SeedPlus contributes to reaching this aim. The project corresponds to integrative plant protection management standards by ensuring an inherent and sustainable water- and plant protection management for efficient emergence even under suboptimal environmental conditions. Lately, especially (bio-)polymers are increasingly used to improve seed germination. However, (bio-)polymers are not yet considered in regulatory requirements of soil protection (e.g. Federal Soil Protection Act).

To address this at an early stage, an evaluation methodology of (bio-)polymers in soils is being developed that considers the ecotoxicological safety as well as the environmental fate of the substances using a stepwise approach. In the first step miniaturised screening approaches will be used, followed by a validation of the screening tools with standard tests according to OECD guidelines (e.g. OECD 201, 202, 301). Finally, the outcome of the two steps will be proven in lysimeter simulations under environmental conditions.

For ecotoxicity, the impact of the substances on the compartments soil and water is investigated. As terrestrial model organisms, e.g. soil microorganisms (MicroResp<sup>TM</sup>) and earthworms (ISO 17512) were chosen, representing parts of the micro- and macrofauna. Aquatic model organisms of different trophic levels, i.e. the green algae *Raphidocelis subcapitata* (OECD 201), the invertebrate *Daphnia magna* (OECD 202) and zebrafish *Danio rerio* (omics approaches) will be used. To consider effects on degrading bacteria in sewage sludge the inhibitory effect on *Vibrio fischeri* according to ISO 11348 will be observed. In addition, the environmental fate of the substances will be determined in sewage sludge (OECD 301) and soil (OECD 307) test systems. <sup>14</sup>C-labelling will be used to trace the degradation of the polymers. Possible degradation products of the polymers will be identified by using substance-specific analytics, e.g. gel permeation chromatography and pyrolysis gas chromatography/mass spectrometry, to develop new analytical end points.

First results of the test miniaturisation for ecotoxicological testing and environmental fate will be presented at the conference.

#### **4.13.P-Th341 Environmental Fate of Water-Soluble Polyethers in Soils: Analytical Method Development, Impact of Polymer Properties, and Implications for Fate Assessment**

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Polyethers present in household products, such as polyethylene glycol (PEG), polypropylene glycol (PPG), and their copolymers, may be released to surface waters from wastewater effluent or to soils from agricultural irrigation and sludge application, and have been previously identified as potentially posing environmental risk. However, like many water-soluble polymers (WSPs), environmental fate data are scarce and standard fate test methods developed for smaller molecules may not be directly applicable. In this study we developed an HPLC-MS method to characterise individual homologues of low molecular weight polyethers (PEG-9 and PPG-7) that had previously been prioritised from household products. OECD-type sorption studies were then used to characterise the sorption behaviour of the polymers in a range of soil types. Polyethers of varying functionality and molecular weight were studied in order to assess the impact of polymer properties on sorption, with characterisation of individual homologues allowing analysis of mixture effects and testing of the applicability of established test methods.

Relatively low levels of sorption were observed for PPG, along with increased levels of sorption with increasing polymer chain length as has been observed previously for PEG. In addition, longer chain lengths were shown to sorb preferentially to soils at high concentrations, occupying sorption sites in place of shorter homologues. Bulk analysis of polymer mixtures during testing



may therefore underestimate sorption of high molecular weight components, and overestimate sorption of low molecular weight components, which may be important in polymer fate studies and risk assessment. The PEG studies are ongoing. The findings of the present study provide insight into the extent that polyether functionality, molecular weight, and molecular weight distribution may influence fate, with implications for risk assessment and grouping. This work also presents a case study for application of established test methods to WSPs.

#### **4.13.P-Th342 It's a Challenge: Determination of Physical Chemical Properties of Polymers**

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As part of the EU's Green Deal and Chemical Strategy for Sustainability it was recently decided to extend the REACH registration to polymers. For the identification and registration of polymers requiring registration (PRRs) a set of criteria need to be determined. For small molecules, data requirements for registration include several physical chemical properties, such as water solubility, surface tension, and partition coefficient (n-octanol/water), as these often form the basis for ecotoxicological and toxicological studies. To generate analytical data on these parameters in a consistent and comparable way, different OECD guidelines are available. Transitioning from defined molecules to polymers, however, is more complex as polymers are inherently characterized by distribution of chain lengths, chemical compositions, end-groups, or architectures. Therefore, the applicability of the existing guidelines on polymers is limited and involves the risk of generating unreliable data with detrimental impacts on risk assessments.

The development of standardized methods for physical chemical characterization is essential, since already the sample preparation has been shown to have a huge impact, e.g. on the water solubility/extractability. Current results and challenges obtained while applying the existing guidelines (OECD 115, 117, 120) on representative polymers are shown and discussed in this poster. In addition, alternative approaches more suitable for polymers are presented. However, it needs to be taken into account, that there is a wide variety of polymers (liquid, viscous, dispersions) on the market, which requires a broad applicability of the methods. Finally, it must be emphasized that in the analysis of polymers there is always a risk of impurities influencing the obtained results. All in all, there are still numerous aspects to be clarified on how robust physical chemical properties of polymers can be determined, to generate a reliable basis for registration and risk assessment of polymers.

#### **4.13.P-Th343 Modernizing the Assessment, Regulation and Management of Environmental Hazards of Polymers**

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In the recent years, a considerable amount of research and policy attention has focused on understanding and mitigating environmental impacts caused by (micro)plastics. At the same time, the environmental safety of polymers, a much broader group beyond just the components of plastics, has received much less attention. In fact, the fundamentals of current approaches to assessing, regulating and managing polymers were developed in the early 1990s, and little has changed since then. Currently, the European Union is developing a regulatory proposal to initiate registration of (selected) polymers under REACH. This offers a unique opportunity to modernize polymer assessment schemes and start collecting the missing information to close scientific and policy gaps. To inform the ongoing discussions, we performed a critical appraisal of recent science advances on the environmental fate, exposure and effects of polymers, which allowed us to identify several opportunities for improving current approaches to regulatory assessment and management of polymers. Four major aspects require more attention in the regulation of polymers: (i) increasing transparency about chemical identities, physical characteristics and grouping approaches for in-use polymers; (ii) improving understanding of the environmental fate of polymers and materials composed of polymers across size and density categories and exposure profiles; (iii) enabling a more comprehensive assessment of the environmental hazards of polymers, taking into account the effects of degradation and weathering as well as the actual uptake, long-term toxicity, and geophysical impacts; and (iv) incorporating the production volume and use/release patterns in determining regulatory data and testing requirements. Transitioning toward a toxic-free and sustainable circular economy will likely require additional policy instruments to reduce the overall complexity and diversity of in-use polymers and polymeric materials. While using the REACH proposal as an example, our analysis has focused on individual assessment criteria that are used in many jurisdictions worldwide, and is therefore broadly relevant for scientists, regulators and other stakeholders working on polymer assessment and management.

#### **4.13.P-Th344 Screening of Polymers for Interface-activity with Lipid Layers**

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Studying the interaction of chemicals with assays of the key initiating event enables a screening for potential hazard via adverse outcome pathways (AOP). Here we focused on the screening of both soluble ("functional") polymers and insoluble, solid polymers (micro- and nanoplastics) with regard to the published AOP of Halappanavar et al. (Small 2021, 17, 2007628). The screening is equally relevant to the criteria of the US-EPA concept of "poorly soluble low toxicity PSLT polymer" and to the EU concept of "polymers requiring registration PRR".

We have implemented three methods for the preparation and characterization of lipid layers, functioning as a model of a) a living cell membrane (DOPC phospholipid, relevant both for environmental species and for humans) and b) the alveolar lining fluid (DOPC, DPPC, DPPG phospholipid, relevant to pulmonary species, incl. humans). The assays detect the interaction of the lipid layers with different chemicals: Firstly, with the quartz-crystal microbalance with dissipation monitoring (QCM-D), where we have prepared a supported lipid bilayer (SLB) at the solid/water interface. And secondly, with interfacial rheology, where a lipid layer is prepared at the water/air interface of a drop. Both preparation methods were successfully implemented. The presence of a

SLB was confirmed by other surface-sensitive methods and the lipid layer at the drop surface was characterized yielding very good agreement with the literature. And lastly, with analytical ultracentrifugation (AUC), which allows to test the interaction between unilamellar lipid vesicles via the integration of the test item or bridging of vesicles. In a first step, chemicals were tested with all three methods, and we see agreement regarding the severity of interaction of chemicals with the lipid layers. Controls included inorganic natural particles (Kaolin), and small-molecule interface-active molecules (surfactants, Triton X), and finally PLGA polymer, which is approved by the US-FDA as carrier in pulmonary delivery of medical actives.

We categorize the tested polymers by their characteristic interaction, or lack of interaction. At the same time, interface activity is essential for many polymers to achieve the required performance in the intended use, e.g., in cleaning, textile washing, cosmetics, medical formulations, waste water treatment flocculation, etc. Trade-offs must be balanced in a safer-by-design approach.

#### 4.14 Tire Wear and Microrubber Particles – Problems to Solution

##### 4.14.T-01 Mapping Our Way Towards an Integrative, Tire Microplastic Mitigation Strategy in a Multi-stakeholder Context

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Tire microplastics, such as tire wear particles, are an important source of microplastics in the environment. This raises the question how to effectively and efficiently mitigate the environmental release of tire microplastics. Assessing whether a mitigation intervention is appropriate requires insight into the environmental sources, pathways and consequences of tire microplastics, but also into the tire's lifecycle, the supply chain, the stakeholders involved and potential socio-economic consequences of interventions. This study provides a first step towards the development of an effective mitigation strategy based on an integrative multi-stakeholder approach.

The aim of this study was to map the tire microplastic emissions along the tire's lifecycle. Thus, providing a systematic overview of the processes associated to the tire's lifecycle and how they relate to microplastic emissions, including the identification of and collaboration with relevant stakeholders. Stakeholder involvement was integrated to utilize their knowledge and expertise and to build trust for further collaboration necessary to ensure that the mitigation strategy is rooted in reality with sufficient support. To this end, the supply chain was mapped and translated into a material system analysis with the Netherlands as geographical location and 2021 as the year of interest. This resulted in the quantification of tire and tire microplastic streams along the supply chain. The total initial release of tire microplastic emissions estimated in this study was 22,593 t/y (74% by tire wear and 26% by infill). Part of the initial emissions are never release to the environment, e.g. due to entrapment in asphalt. The emissions that are emitted to the environment were estimated at 11,163 tonnes, of which 83% to soils and 17% to surface waters.

The presented mapping method can be applied to other regions to acquire insight into the link between the tire's lifecycle and tire microplastic emissions. Actors, such as regulators and the tire industry, can apply these results to initiate changes by better understanding their own role and how emission sources relate to processes in the supply chain.

As this study only provides the first stage, additional steps are needed in order to reach an effective mitigation strategy. Future research steps include the coupling of the presented mapping results to a fate transport model and further integration of stakeholder collaboration and environmental modelling.

##### 4.14.T-02 Online Thermogravimetric-gas Chromatography Mass Spectrometry Coupling for a Fast Detection and Quantification of Tyre Wear Particles in Road Dusts

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This work describes the use of thermogravimetric analysis coupled to gas chromatography-mass spectrometry (TGA-GC/MS) to carry out both qualitative and quantitative analysis of tyre rubbers in road dust samples. Currently, no methodology allows for identification of rubber particles in environmental samples without sample preparation, as is demonstrated in this work. The work described here provides several advantages such as the ability to collect direct quantitative data on samples through thermogravimetric analysis and the use of a less polar GC column phase, allowing for better separation of degradation products and much faster run times. This work revolves around using the TGA-GC/MS system in two modes of operation to understand the thermal degradation of both sidewall and tread of passenger car tyres as they are subjected to controlled temperature ramping: (1) conventional TGA-GC/MS; and (2) PyroTGA-GC/MS, which involves pyrolyzing the sample at an elevated temperature and again measuring the evolved gases with GC/MS.

The presentation will show TGA-GC/MS analysis of tyre rubber from home-made standards and PyroTGA-GC/MS analysis of road dust from environmental samples collected next to the M25 and M34 in United Kingdom and centre London. This research provides understanding in the thermal degradation of tyre tread and sidewall rubber, allowing for a differentiation between the two, based on markers of styrene-butadiene rubber (SBR) and natural rubber (NR) also seen in other work. Furthermore, going beyond conventional pyrolysis-GC/MS, PyroTGA-GC/MS is used to identify the presence of tyre rubber in road dust samples. The presence of degradation products of vulcanising agents and other additives, such as SO<sub>2</sub> and aniline, is also seen in the chromatogram. The thermogravimetric analyzer provides direct quantitative information on the polymer content in the road dust samples. In the road samples analysed, the polymer content was ca. 3.1%. This work proposes a new method whereby tyre rubber and road dust samples can be analyzed with minimal sample preparation, providing information on the presence of rubber

particles, degradation products and additives, in environmental samples as well as information regarding thermal degradation patterns of tyre rubbers.

#### **4.14.T-03 Tire Wear Particles and Chemicals: Emissions, Runoff, and Presence in San Francisco Bay, USA**

**Kelly Moran<sup>1</sup>, Alicia Gilbreath<sup>1</sup>, Miguel Alexander Mendez<sup>1</sup>, Ezra Miller<sup>1</sup>, Edward P. Kolodziej<sup>2</sup>, Diana Lin<sup>1</sup> and Rebecca Sutton<sup>1</sup>, (1)San Francisco Estuary Institute, (2)University of Washington**

Every vehicle on the road sheds tiny particles from its rubber tires into the environment. As they disperse into the environment, tire particles convey tire tread ingredients into the air, into runoff, and eventually into surface waters. In most of the USA, including California's San Francisco Bay area, urban stormwater runoff usually carries tire particles from roads directly to surface waters, through separate storm drain systems designed to prevent flooding and avoid sanitary sewer overflows. San Francisco Bay area monitoring found that tire particles are the single most common microplastic flowing into the Bay and that after storm events, tire and roadway contaminants reach detectable concentrations in Bay water near stormwater discharge locations as well as in the center of the Bay, even with dilution from mixing. Furthermore, 6PPD-quinone reaches concentrations that may be of concern for endemic salmonids both in the creeks during storms and within near-shore Bay water post-storms. To inform future monitoring design and mitigation strategies, we estimated tire wear emissions in the San Francisco Bay area. Using tire sales and measured tire wear particle emissions data from reliable sources and local vehicle miles traveled data, we have estimated annual total and per-capita vehicle tire particle emissions. We used two approaches: (1) the emission factor/mileage method and (2) the sales/tread-loss method. We compared the estimated annual tire wear particle emissions to monitoring-based estimates of total annual tire-wear particle flows into San Francisco Bay to obtain insights into the transport of particles between on-road emissions and aquatic habitat.

#### **4.14.T-04 Deep Dive Into the Chronic Toxicity of Tyre Particle Mixture and their Leachates to the Planktonic Crustacean *Daphnia magna***

**Paul Benjamin Boisseaux<sup>1</sup>, Cassandra Rauert<sup>2</sup>, Pradeep Dewapriya<sup>2</sup>, Marie-Laure Delignette-Muller<sup>3</sup>, Robyn Barrett<sup>4</sup>, Lee Durndell<sup>4</sup>, Florian Pohl<sup>5</sup>, Richard Thompson<sup>6</sup>, Kevin Thomas<sup>2</sup> and Tamara Galloway<sup>7</sup>, (1)Biosciences, Exeter University, United Kingdom, (2)University of Queensland, Australia, (3)Université de Lyon 1, France, (4)School of Geography, Earth and Environmental Sciences, University of Plymouth, United Kingdom, (5)University of Plymouth, United Kingdom, (6)Plymouth University, United Kingdom, (7)University of Exeter, United Kingdom**

This study compares the chronic toxicity of tyre-tread particles and their leachates toward *Daphnia magna*. Sigmoidal survival curves allowed the calculation of LCx(21d) for both, particles and their leachates. Particle mixture induced hormesis response on the reproduction, while leachates severely decreased it (as described by the 21d-benchmark doses). At environmentally relevant concentrations, tyre particles triggered an hypertrophy of daphnids tail, while their leachates increased body length and width. A clear dose-dependent tyre particle ingestion in daphnids' gut was observed. A slight alteration of haemoglobin content and thiol proteins was suggested (trends only). Tyre-tread microparticles were characterised by stereomicroscopy (size distribution and concentrations) as well as scanning electron microscopy. The leachates characterization revealed a metal predominance of Zinc, Titanium and Strontium. Plenty of organic chemicals were found, with a total of 50 molecules shared across the 5 tyres used in the mixture. Many of these chemicals are known to be very toxic and will put ecosystems under strong chemical pressure. Overall, this study demonstrates that tyre-tread particles are chronically lethal to *D. magna* at worst-case environmental concentrations, while tyre concentrations closer to environmental reality alter strongly the reproduction and morphology development of daphnids. In conclusion, this study provides an overview of why tyre particles are hazardous pollutants of particular concern.

#### **4.14.T-05 The need For Environmental Regulation of Tires: Challenges and Recommendations**

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The interest in tire wear particles (TWPs), generated from abrasion of tires, have gained traction over the past few years, both in regards to quantifying particulate emissions, leaching of different compounds, toxicity, and analytical methods. The life of a tire, from cradle to end-of-life, crosses over different scenarios during its lifetime and transcends environmental compartments and legislative areas, underlining the need for a collective approach. Sustainability for a tire encompasses the use of raw materials, recycling of raw materials, circular economy and material sourcing. The tire industry is currently making significant efforts towards a greener and more sustainable production considering reduction of CO<sub>2</sub>-emissions, recycling, material sources and implementing the use of biomass from plants rather than oil-derived alternatives. In this paper, we aim to analyze and discuss the need for environmental regulation of tires in order to provide a series of targeted recommendations for future legislation. Our study shows that the numerous regulations related to tires focus on chemicals, manufacturing, raw materials, use of tires on roads, waste handling, safety and polycyclic aromatic hydrocarbons (PAHs) in different life cycle stages of a tire. However, none directly addresses the contribution of TWPs to the environment. Despite the overall good intentions of the existing regulations, there is a lack of focus on the compounds that partition from the tire and disperse in the environment, their mixture effects, and the trans-formative products from the parent compounds in the environment. Therefore, a renewed focus is needed on risk assessment of complex mixtures like TWPs. Thus, transparency in regard to use of chemicals in TWP, mixtures, minimization of emissions, and capture of particulate pollution should be a priority

#### 4.14.P Tire Wear and Microrubber Particles – Problems to Solution

##### 4.14.P-Th345 Fast Detection and Quantification of Tyre Wear Particles in Road Dusts via Online Coupled TG-GC-MS

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The generation of tyre wear particles (TWP) by abrasion of vehicle tyres against road surfaces has gained global attention due to potentially being the largest source of microplastic contamination in the environment. Currently reported experimental data disagrees with these estimations of TWP emissions in the environment. This discrepancy maybe due to a low environmental availability or, an inadequacy of detection due to lack of standardisation in the methodologies and the fact that TWP do not respond well to common microplastic separation and detection techniques. Here we present a method for detecting and quantifying TWP in road dusts by online coupling of thermogravimetric analyser (TGA) and gas chromatography mass spectrometry (GCMS) for a fast, high-throughput, automated analysis. Various thermal degradation products of rubber elastomers were identified in different car tyre brands as common markers for TWP. Evaluation of the treads and sidewalls showed different rubber compositions that could potentially indicate the origin of the rubber fragments – a tyre wear or tyre blowout. Subsequent analyses of road dust samples collected from two major highways in the United Kingdom confirmed the presence of TWP and were quantified as percentage mass of the environmental sample. Historical road dust samples collected in 2009 from various points through London congestion zone were also investigated. This approach requires minimal to no sample preparation eliminating the risk of sample loss or contamination and reducing variability in data amongst researchers when different sample preparation methods are used.

##### 4.14.P-Th346 Infrared Measurement of Tyre Wear Microplastics

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The scientific attention on tyre and road wear particles (TRWPs) has increased notably in recent years as road traffic has proved to be an important contributor to the release of microplastics (MPs) to the environment. More than 1 million tons of tyre degradation particles per year are estimated for European roads. There are also significant differences between tyres used in cars, composed mainly of synthetic rubber (SBR or BR), and those used in planes, trucks and busses, which use more natural rubber. Further, the original composition of TWPs evolve with the temperature when driving, chemical/physical reactions with the asphalt compounds, friction, etc. All this make the characterization of microplastics from tyre wearing difficult. In this work several forms of reflectance measurements are studied in order to select the most suitable one for current monitoring purposes; namely: macro attenuated total reflectance (ATR), micro reflectance spectrometry and micro imaging with a quantum cascade laser-based IR system. The spectra from the different instruments will be compared to ascertain the chemical information they offer and how viable they are for the analysis of actual field samples.

##### 4.14.P-Th347 Aging of Tire and Road Wear Particles – Results of a Lab Study

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Tire and road wear particles (TRWPs) are emitted into the environment where cars are driven on road surfaces. Global emission was estimated to amount to approx.  $6 \times 10^6$  t/a and to  $1.3 \times 10^6$  t/a in Europe. It is largely unclear how long TRWPs remain in the different environmental compartments after creation and distribution, how long it takes for them to be degraded and how their properties change during aging in the environment. This study investigated changes in physical and chemical properties of TRWPs by different environmental stressors, namely elevated temperature, sunlight and mechanical stress, using a combination of analytical techniques. Contrary to tire particles (TP) which are often used in lab experiments, TRWPs are heteroaggregates of tire particles with road material. Aging experiments under controlled conditions in the laboratory were performed with TRWPs to simulate real world aging. Physical and chemical properties are observed using a range of analytical techniques. Particle size distribution (laser obscuration), morphology (dynamic image analysis), particle density (sequential density separation), as well as around 20 organic constituents (extractables) and the release of organics into water (leachables) by LC-MS.

No marked changes were observed for size and density distributions of TRWPs by the aging experiment. However, mechanical stress appeared to induce a loss of mineral incrustations from the particles surface.

Changes in the organic chemical composition by exposure to elevated temperature and sunlight were much more pronounced. Sunlight exposure had stronger effects on the chemical composition than elevated temperature. These changes in the chemical composition of the particulate phase were largely reflected in the respective leachate, though modulated by ongoing transformation for some of the compounds.

The results of this lab aging study suggest that TRWPs, although being heteroaggregates, are comparatively stable particles against mechanical stress. First signs of physical breakdown may be the loss of mineral incrustations from the surface. However, exposure to elevated temperature and sunlight has strong effects on organic chemicals, the extractables and leachables of TRWPs and should be considered further also in studies on effects of TRWPs.

##### 4.14.P-Th348 Leaching of Organic Compounds from Microrubber Under Conditions Simulating the Sea Surface and the Deep Sea

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Microrubber (MR) can contain high amounts of additives, such as vulcanization agents (e.g. 1,3-diphenylguanidine (DPG) and benzothiazoles), antioxidants and antiozonant (e.g. N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6-PPD)) and heavy metals like zinc, which can leach out. Here, we present results from two experiments aiming to investigate the leaching of organic compounds from MR under conditions simulating (1) the sea surface and (2) the deep sea. Different MR materials were tested: (i) cryo-milled tire tread (CMTT), (ii) 'virgin' crumb rubber (VCR) and (iii) weathered crumb rubber (WCR) which consisted of VCR that had been immersed in the sea for 12-18 months prior to the experiments. The MR (1 g L<sup>-1</sup>) was added to filtered seawater and exposed to the following conditions: (1) UV irradiation (300 W m<sup>-2</sup> at 300-800 nm, 22±2°C) vs dark controls, for max. 336 h simulating 31 days in the environment or (2) high hydrostatic pressure (200 bar) vs atmospheric pressure conditions, for max. 14 days. Leachates were then analyzed for organic compounds using RPLC-HRMS and for dissolved organic carbon (DOC).

We observed (1) a considerable increase in DOC concentrations in samples exposed to UV irradiation compared to dark controls for all materials. This trend is generally reflected by the leaching of vulcanization accelerators, such as DPG and benzothiazole. In contrast, 6-PPD quinone (6-PPDQ), one oxidation product of 6-PPD, showed a 2-5 fold increase in dark conditions compared to light conditions in CMTT (max. 0.5 µg L<sup>-1</sup>) and VCR (max. 0.2 µg L<sup>-1</sup>) samples. In WCR samples, however, 6-PPDQ concentrations increased steadily in light and dark conditions, but with significantly lower values overall (max. 0.03 µg L<sup>-1</sup>). (2) Preliminary results indicate higher DPG concentrations for CMTT under high hydrostatic pressure conditions compared to atmospheric pressure conditions (max. 71 µg L<sup>-1</sup> vs 47 µg L<sup>-1</sup>, respectively), whereas benzothiazole (max. ~160 µg L<sup>-1</sup>) and 6-PPDQ (max. ~1.5 µg L<sup>-1</sup>) exhibited similar concentrations in the leachates of both pressure conditions.

Our study showed that MR particles release organic compounds under all tested conditions, with patterns dependent on compound classes and exposure conditions. After ≥12 months in the environment, WCR still released a variety of organic compounds, often in concentrations similar to those of VCR. This suggests a long-term potential of MR to release additive compounds into the marine environment.

#### 4.14.P-Th349 Chemical Evolution of Metals During Tire Weathering in a Marine Environment

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Tires are a chemically complex and heterogeneous mixture, consisting of approximately 5–10% additives needed for tire production, stability and durability. Thus, a tire can be composed of as many as 200 different additives that may be released into the aquatic environment through leaching.

Tires contain several potential toxins, including the heavy metals zinc, cadmium and lead. Zinc oxide is added as an activator during the vulcanization process used to reinforce rubber, and cadmium is mineralogically associated with zinc. Lead is present in the steel fibers used to reinforce the rubber. Other heavy metal elements in tire tread are Mn, Fe, Co, Ni and Cu.

As part of the MicroplastiX project's research activities, in-situ experiments were carried out simultaneously in 5 port sites in Europe and in an extra location in Brazil. The experiment aims to assess degradation, fragmentation and weathering of different polymers under environmental conditions.

In this work, the tire weathering study to monitoring changes in its metal additives composition is presented. The weathering experiment has been carried out during the summer, autumn, winter and spring of 2021-2022 in a sport port in Ares, A Coruña, Spain. Subsamples were analyzed at each season at 0, 30, 60 and 90 days of exposure. 15 metals were simultaneously measured by ICP-MS after microwave acid digestion.

Seasonal variations were found, being the summer season the one with the highest concentration values for some of the metals analyzed. Ca, Fe, Mg and Zn are the metals analyzed that have been found in the highest concentrations; on the other hand, Hg was not found at any time in any of the stations. In addition, the time trend of the metals was analysed individually, both in terms of leaching in some of them and adsorption from the marine environment to the tire for others.

#### 4.14.P-Th350 Biodegradation Studies on Tire Tread Particles: A Comprehensive Investigation on Leachates Composition

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Tire wear particles (TWPs) in aquatic environment act as a source of various organic pollutants released by leaching. These contaminants consist of a complex mixture of tire additives needed for tire production and for stabilizing them during use, such as vulcanization accelerators and antioxidants. Biodegradation studies on TWPs and their related compounds are very scarce, with a major focus on single compounds than the whole pool of substances in the leachates, that may interact with each other. Here, we report the first biodegradation study on both TWPs and their leachates performed with a cryo-milled tire tread (CMTT) particles mixture dispersed in a mineral medium with active sludge. Both leachates, first in presence and then in absence of the CMTT particles acting as a source, were investigated. Furthermore, a surface particle characterization was performed on the CMTT particles after the biodegradation.

The CMTT was prepared by mixing together 20 types of new, used and aged tires. The mineral medium and the active sludge collected from a waste water treatment plan (WWTP) were prepared according to the OECD 301 guidelines. The CMTT particles and sludge concentration used was 4 g L<sup>-1</sup> and 0.3 g L<sup>-1</sup>, respectively. The Eyetechn Combi particle analyzer and the Zeiss Merlin

VP scanning electron microscope (SEM) were used for the size and shape characterization of the particles, while the leachates were analyzed by ultra-performance liquid chromatography time-of-flight mass-spectrometry (UPLC-TOF-MS). As a result, the sludge inoculum had a key role in both CMTT particles and leachates. On the surface of the CMTT particles a biofilm formation was visible. The lower DOC concentrations found in the aqueous phase confirmed that biodegradation of many leachables took place, in particular for our compounds of interest. Most of the vulcanization accelerators and antioxidants were biodegraded by the sludge, promoting the formation of new transformation products. Furthermore, the combination of both experimental parts performed with and without CMTT particles showed a different biodegradability behaviour of the leachates when the source is present, suggesting that the biofilm played a relevant role in the particles leachability.

#### **4.14.P-Th351 Laboratory Studies on the Degradation Behaviour of Tire Wear Particles in Aquatic Ecosystems**

**Robin-Macmahon Bähre<sup>1</sup>, Mirko Wiebking<sup>2</sup>, Hannah Behnse<sup>2</sup>, Jacek Lecinski<sup>2</sup> and Hans-Josef Endres<sup>2</sup>,** (1)*Mechanical Engineering, Leibniz University of Hannover, Institute for Plastics and Circular Economy, Germany,* (2)*Leibniz University of Hannover, Institute for Plastics and Circular Economy, Germany*

Tire wear particles (TWPs) are among the biggest sources of microplastic pollution. A considerable portion of the contaminant ends up in surface waters, which leads to unpredictable consequences for sensitive aquatic ecosystems. In this work TWPs were generated in a tire tread buffing process and subsequently examined in two experiments to investigate the role of different parameters on the degradation behaviour of TWP. Three main influence categories are considered: (i) the material composition of TWPs (e.g. rubber type, anti-aging agents), (ii) the relevant environmental conditions in aquatic ecosystems (e.g. temperature, light availability (UV), salinity, mechanical stress) and (iii) the morphology of TWPs (e.g. particle size, specific surface area). During this study two different experimental setups were used to investigate the degradation behaviour of TWPs. The first is an open microcosm experiment, in which different physical influencing factors (temperature, UV, mechanical stress) are used to simulate a surfzone within a beach environment. For the second approach, manometric respirometry is used to investigate the biodegradation of TWPs for different scenarios via oxygen consumption. To measure degradation processes the study uses accompanying analytical methods like imaging techniques (SEM, particle measurement) as alongside thermo-analytical approaches (TGA, Py-GC/MS).

The potential findings of this work help to determine the fate and impact of aquatic ecosystems that are exposed to TWPs. In order to develop effective mitigation strategies and retention measures, the understanding of the persistence and underlying degradation mechanisms of TWPs must be further investigated.

#### **4.14.P-Th352 Investigation into the Oxidation and Ozonolysis of 6PPD and 6PPD-quinone**

**Rory Mumford<sup>1</sup> and Pete Yeomans<sup>2</sup>,** (1)*Environmental Fate and Metabolism, Smithers, United Kingdom,* (2)*Environmental Fate and Metabolism, Smithers, United Kingdom*

Research into Urban Runoff Mortality Syndrome (URMS) has sought to identify some of the pollutants that are potentially toxic to species present in urban watercourses. Certain species were identified as being particularly sensitive to 6PPD-quinone, a degradation product of a 6PPD, which is one of the most widely used antiozonants present in tires and other rubber products produced and used globally. Literature has suggested that tire and road wear particles (TRWP) are a potential source of 6PPD-quinone in watercourses.

The current study was undertaken to further understand the behaviour of 6PPD and 6PPD-quinone in the solid state when exposed to various concentrations of oxygen and ozone. <sup>14</sup>C-radiolabelled 6PPD and 6PPD-quinone were synthesised to aid the tracking of degradation products throughout the study. The oxidation of 6PPD and 6PPD-quinone were studied at reduced and atmospheric levels of oxygen. The ozonolysis of 6PPD and 6PPD-quinone was studied at environmentally-relevant levels of ozone. In both cases, the effects of the presence or absence of sunlight was studied. The results of the study are discussed in this presentation.

#### **4.14.P-Th353 Investigation into the Hydrolysis of 6PPD and 6PPD-Quinone.**

**Rory Mumford<sup>1</sup> and Graham Crabtree<sup>2</sup>,** (1)*Environmental Fate and Metabolism, Smithers, United Kingdom,* (2)*Environmental Fate and Metabolism, Smithers, United Kingdom*

Research into Urban Runoff Mortality Syndrome (URMS) has sought to identify some of the pollutants that are potentially toxic to species present in urban watercourses. Certain species were identified as being particularly sensitive to 6PPD-quinone, a degradation product of a 6PPD, which is one of the most widely used antiozonants present in tires and other rubber products produced and used globally. Literature has suggested that tire and road wear particles (TRWP) are a potential source of 6PPD-quinone in watercourses.

The current study was undertaken to further understand the behaviour of 6PPD and 6PPD-quinone once they have entered the aquatic environment. <sup>14</sup>C-radiolabelled 6PPD and 6PPD-quinone were synthesised to aid the tracking of degradation products throughout the study. The hydrolytic behaviour of 6PPD and 6PPD-quinone were studied through the environmentally-relevant pH range using the standard OECD TG111 buffers. Because trace elements are known to impact the rates and the routes of degradation in the environment, sterile natural waters were used to assess the influence of alkalinity, hardness, salinity, and trace element content.

Comparison with active natural water was made to assess the biotic effect on hydrolysis and degradation. The results of the study are discussed in this presentation.

#### **4.14.P-Th354 Procedures to Assess Traffic-related Micro- and Nanoplastic Exposure**

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Korinna Altmann<sup>9</sup>, Raymond Pieters<sup>10</sup> and Gerard Hoek<sup>10</sup>, (1)Institute for Risk Assessment Sciences, Utrecht University, Netherlands, (2)A-Life, Vrije University Amsterdam, Netherlands, (3)Utrecht University, Inorganic Chemistry and Catalysis, Department of Chemistry, Netherlands, (4)Chemistry, Utrecht University, Netherlands, (5)BAuA, Germany, (6)Federal Institute for Occupational Safety and Health, Germany, (7)Fraunhofer IMWS - Institute for Microstructure of Materials and Systems, Germany, (8)ENEA Environmental Chemistry Division, Italy, (9)Federal Institute for Materials Research and Testing, Germany, (10)Utrecht University, Institute for Risk Assessment Sciences, Netherlands

There is increasing concern about micro- and nanoplastics (MNP) in the environment. One of the predominant sources releasing MNPs into the atmosphere is motorized traffic tyre wear. The aim of this study was to develop procedures to characterize and quantify potential traffic-related MNP exposure in various urban settings.

4-hr air sampling was performed within Utrecht, the Netherlands, with a potential high contrast in MNPs and other major air pollutants. Measurement sites were a stop-and-go location, highway and city park as urban background location. Using a high volume sampler quartz filters were collected for MNP analysis using pyrolysis- and Thermo-Extraction/Desorption (TED)-Gas Chromatography/Mass spectrometry (GC/MS), other major traffic contaminants were measured using Inductively Coupled Plasma (ICP)-MS. Gold-coated Nucleopore filters were collected and assessed for spectroscopic MNP analysis using Scanning Electron Microscopy coupled with an Energy Dispersive X-Ray analyser and Raman microspectroscopy analysis. We determined PM10 mass concentrations and particle number concentrations (PNC).

Air sampling and analytical procedures were adapted to assess MNPs while avoiding contamination. Analysis of the collected filters is still ongoing. 9 µg of styrene-butadiene rubber (SBR) and 186 µg (poly-ethylene) per half of the stop-and-go quartz filter were found using TED-GC/MS analysis after pre-treatment with density separation, which is a factor 100 above the LOD of pristine polymer measurements. The average zinc and ΣPAH concentrations at the stop-and-go location were respectively 8.95 (7.7) ng/m<sup>3</sup> and 2359 (1972) pg/m<sup>3</sup>, which was 4.7x and 2.7x higher compared to the urban background location. The average PM10 mass and PNC concentrations, together with the major air pollutants, were highest at the highway, with respectively 35.0 (17.1) µg/m<sup>3</sup> and 35,600 (20,900) pg/cm<sup>3</sup>.

Zinc is often used as a marker for tyre wear. Together with the preliminary TED-GC/MS results, this might indicate that the traffic-related MNP contribution was higher at the stop-and-go location compared to the urban background location. Results will be presented on differences in MNP concentrations at these three locations, also supported by data on chemical characterization of the collected samples. The exposure estimates will be used in a human volunteer study, assessing immuno-toxicological effects of exposure to traffic-related MNPs.

#### 4.14.P-Th355 Tire Wear Particles in Soils Along Low Traffic Density Roads

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Recent studies have suggested high concentrations of tire wear particles in road dust and soil along roads with high traffic density. There is, however, less knowledge about the levels of tire wear particles along roads with low traffic density. In this study we have explored the levels of tire wear particles in road-side soils along country roads in Trøndelag, Norway, where the traffic density was between 650 and 12500 vehicles /day and traffic speed between 60 and 80km/hour. Samples were collected from 0m, 1m, 3m and above 6m distance from the road. Samples were collected from the topsoil (0-0.1m) and lower soil layer (0.1-0.2m). Soil samples were analyzed using pyrolysis gas chromatography mass spectrometry for tire wear particles and ICP-MS for metal concentrations (Zn, Ni, Cd, As, Cr, Pb). Levels of tire wear particles varied between different sites, from 2040 to 26 400 mg/kg. These levels are comparable to one previous study of road-side soils with medium traffic density (AADT 36 000), and far exceeds the levels reported in another study with high traffic density (AADT 71 250). This suggests that levels of tire wear particles in soils are less impacted by traffic density. No significant difference was found for samples grouped by soil layer, distance from the road or traffic speed either. The variation of tire wear particles in the soil were further explored by redundancy analysis, where metals measured in the soil samples were also included. Using all the explanatory variables for traffic (speed, traffic density) and soil properties (loss of ignition, soil type, soil layer, drainage profile) had no significant relationship with the tire wear particle levels, however, they explained 42% of the variation found for metals. This study is an important contribution to the knowledge base on tire wear particles in different environments, especially liked to lower traffic densities. It is also valuable to compare results between tire wear particles and other road pollutants that have been studied for decades already, such as metals.

#### 4.14.P-Th356 Vertical Distribution of Microplastics Including Tire Wear Particles in the Air, Sea Surface Microlayer and Underlying Water in Swedish Fjord Systems

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Microplastics (MP) and tire wear particles (TWP), recently included in the MP definition, are omnipresent in the (marine) environment. Still, little is known about these pollutants concerning mass-based concentrations, transport and effects in the marine environment and boundary layer, which covers air, sea surface microlayer (SML) and underlying water (ULW). The SML is a ubiquitously occurring, predominantly organic film with a thickness below 1 mm.

Air, SML and ULW (1 m depth) samples were collected simultaneously with a remote-controlled research catamaran. Three Swedish fjord systems with different anthropogenic influences (urban, industrial and rural areas) were chosen for sample collection with the aim to learn more about vertical MP transport and potential enrichment in the SML.

Identification and quantification were conducted with pyrolysis-gas chromatography-mass spectrometry based on polymer specific backbone-related clusters. These polymer clusters were indicated by the prefix “C” and included amongst others polyethylene (C-PE), polypropylene (C-PP), polyethylene-terephthalate (C-PET), car, and truck TWP (CTT&TTT). All air and water samples contained MP. Most occurring polymer clusters in the air were CPET and CPC. Concentrations in the air reached up to 49 ng/m<sup>3</sup> air in the fjord in the urban area. Least polluted was the fjord in the rural area. This also applied for the water samples. The fjords in urban (8.5 µg/L SML and 6.0 µg/L ULW) and industrial environments (6.6 µg/L SML and 7.3 µg/L ULW) were more polluted than the rural fjord system (1.8 µg/L SML and 2.0 µg/L ULW). Water samples were predominantly polluted by C-PMMA, CTT and C-PET. C-PE, C-PP, C-PET and CTT were accumulated in the SML while C-PMMA and C-PC were more present in the ULW.

#### **4.14.P-Th357 Determination and Mitigation of Microplastic and Pollutant Emissions from Synthetic Turf Sports Fields**

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Synthetic turf is becoming increasingly popular. However, the use of plastics as a surface covering is also potentially associated with the risk of a possible release of microplastics and other chemicals into the environment.

To assess and determine the infill losses released into the environment, approximately ten different artificial turf pitches are under investigation. The emissions of microplastics and chemicals of concern will be evaluated in water, soil in the surroundings of the sport fields as well as air using TED-GC-MS analysis. To ensure correct identification and quantification of the polymers, an optimisation of the analytical parameters will be carried out before the sampling campaign.

The effectiveness of particle removal of infill losses from new and old sports facilities, taking into account factors such as ageing, weather conditions and frequency of use, will be analysed as part of the project. Furthermore, a new filtration technology for particle separation that is installed directly on the playing field will be evaluated at Sittensen, Germany.

After completion of the investigations, the loads of microplastics and other water-relevant pollutants will be calculated.

#### **4.14.P-Th358 Can Tire Wear Markers Be Used to Estimate the Load of Microplastics Derived From Tires in River Bed Sediments?**

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Tire wear particles (TWP) have been proposed as a significant contributor to microplastic contamination of freshwater environments through road run-off and are predicted to concentrate in sediments given their relatively high density. Estimates have been made as to the emissions of this material to freshwaters, but given the complex nature of this composite material, these must be grounded on robust, reproducible and quantitative methods.

In this pilot study we attempted to establish a reproducible method for the analysis of TWP in sediment matrices using Gas Chromatography-Mass Spectrometry (GC-MS). TWPs offer a unique challenge compared to other microplastic materials due to their optical properties, which prevents their detection in complex matrices using the more traditional method of Fourier Infrared Transformation, FTIR. Importantly, GCMS offers the potential to quantify mass of TWP, a key requirement for parameterising and ground truthing material flow assessments and models of microplastic fate in the environment. Quantification of TWP will be compared to other microplastic loads in select sediments (FTIR) to provide insight into the relative contribution of this source of pollution in sediments.

We used 6-PPD (N-phenyl-N'-(1,3-dimethylbutyl)-p-phenylenediamine) as a marker to assess the presence of TWP. We established a method where TWP were extracted from sediments using microwave digestion followed by clean-up steps before analysis on GCMS. For quality purposes, a surrogate compound was used to spike the samples prior to extraction to demonstrate consistent recovery of 6-PPD standards from sediment matrix. Quality assurance and control in new methods is essential, so the approach and results from this effort will be reported and general lessons summarised.

Given the high heterogeneity of sediments, any method employed must be sufficiently sensitive to measure relevant differences between sampling sites. With this in mind, the design of river sediment sampling was such that the spatial heterogeneity of sediments could be interrogated using two exemplary sites, one on the Thames River, and the other in the Greater Manchester catchment, UK. In doing so, not only is a method of a relevant marker for TWP from complex sediment samples presented, but also insights into the degree of heterogeneity inherent to river sediments. This knowledge is essential to underpin the design of sampling and monitoring campaigns for this pollutant in the future.

#### **4.14.P-Th359 First Screening of Volatile Methylsiloxanes in Crumb Rubber**

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One of the possible “destinations” of recycled tyre rubber are synthetic turf sports’ facilities, where this material is commonly applied as infill, in the form of small granules – crumb rubber – now included in the family of microplastics (<5 mm). In spite of the benefits that this approach carries from a circular economy perspective, several public health concerns have been raised regarding a recurrent contact with crumb rubber during physical exercise, with several studies having detected the presence of potentially hazardous chemicals in this matrix, including PAHs, plasticizers and heavy metals, among others. The main goal of



this work was to evaluate for the first time the presence of volatile methylsiloxanes (VMSs), a class of ubiquitous anthropogenic contaminants, in crumb rubber. A total of 126 samples were analysed – 120 field samples (108 of crumb rubber and 12 of other infill materials) and 6 commercial unused samples. Seven VMSs were analysed – three linear (L3, L4, L5) and four cyclic (D3, D4, D5, D6). The extraction protocol employed consisted of an ultrasound-assisted extraction, using a 1:1 (v/v) mixture of n-hexane and dichloromethane, with a QuEChERS clean-up methodology. The quantification of the extracts was carried out by gas chromatography coupled with a mass spectrometry detector (GC-MS). The method detection limits ranged from 0.006 to 4.46 ng.g<sup>-1</sup> (for D5 and D3, respectively). VMSs were detected in all samples, in total concentrations ranging from 10.29 to 5,089 ng.g<sup>-1</sup>. D3 and the linear compounds were not detected in any of the field samples – only in commercial crumb rubber. The mean concentration of commercial samples (976.1 ± 2,016 ng.g<sup>-1</sup>) was 17.6 times higher than field crumb rubber (34.50 ± 16.09 ng.g<sup>-1</sup>), and cork was the only infill material with a lower mean concentration than crumb rubber (27.14 ± 31.22 ng.g<sup>-1</sup>). From these results, oral and dermal exposure doses were estimated for assiduous users of synthetic turf football fields: young players (6-11 years old), adult players and turf maintenance workers. “Worst-case scenario” oral doses ranged from 8.23 to 21.3 ng.kg<sup>-1</sup>.year<sup>-1</sup> and dermal doses from 0.598 to 0.898 ng.kg<sup>-1</sup>.year<sup>-1</sup>.

#### **4.14.P-Th360 Environmental Dispersion and Fate of TWP in the Environment: The LEON-T Approach: Towards Reliable and Comparable Data**

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In the past decade, microplastics have drawn much attention. Tyre wear particles (TWPs) are estimated to be a large source of microplastics (MPs) emission. To understand their impact on the environment better, reliable and comparable data on their distribution, physicochemical properties and fate is necessary. This data is key to allow balanced legislative measures to limit the spread of these particulate emissions to be adopted. Several models exist that estimate the dispersion of particulate matter from the road into the wider environment (air, soil, water). However, these models lack calibration data for the actual particle size distribution of the emitted particles and for the quantification and possible transformation of particles in the different environmental compartments. In the European project LEON-T (Low particle Emissions and Low Noise Tyres) thermo-analytical and microscopic techniques and methods have been assessed and improved, and a methodological framework was set up for measuring TWP to allow reliable quantification and size selective characterization of TWP in environmental matrices. For the evaluation of thermo-analytical techniques, a selection of environmental samples was analyzed using different methodologies described by Tromp (TNO), Eisentraut (BAM) and Unice (ISO/TS 21396 & 20593). Furthermore, to gain more insight in the contribution of TWP to microplastic distribution in the environment selective field measurements were performed based on identified data gaps from existing field data. These monitoring campaigns involved measurements of TWPs in air, atmospheric deposition, surface water, sediment and soil at varying distances from highways, urban and rural roads. To gain insight into the physicochemical properties, photochemical transformation and degradation processes, road simulator tests were performed to collect tyre wear material for additional accelerated UV aging experiments. The collected samples were analyzed using a complimentary toolbox of techniques, i.e., cascade-filtration, pyrolysis gas-chromatography with tandem mass spectrometry (TGA-TDU-GCMSMS) and high-resolution scanning electron microscopy in combination with energy dispersive X-ray analysis and cathodoluminescence (FEG-SEM-EDX/CL)). The obtained data will be used to validate the new LEON-T emission model to be developed to further estimate the fate of TWP in the environment (air, water and soil) and inform policy on effective measures to reduce TWP emission.

#### **4.14.P-Th361 Microplastics Pollution from Communication Routes: Detection of Tire Particles in Environmental Samples** *Katarzyna Styszko<sup>1</sup>, Jagoda Worek<sup>1</sup>, Xymena Badura<sup>2</sup>, Anna Bialas<sup>1</sup>, Joanna Chwiej<sup>1</sup> and Kamil Kawon<sup>1</sup>, (1)AGH University of Science and Technology, Poland, (2)Oil and Gas Institute, National Research Institute, Poland*

Microplastics are one of the largest pollutants in the world. Since the 1970s, it has already existed, but there has been no better alternative than plastic. It is related to easy and cheap production, high availability, and specific properties of plastics, such as plasticity, chemical resistance, and lightness. An important issue related to dust emission is the microplastic of tyres and brake blocks. Typically, two estimation methods are used to quantify tire wear. The first concerns the application of the emission factor to the vehicle per kilometer. The second method is based on estimating the degree of loss of tire mass. The emission masses per capita are estimated to be in the range of 0.2 to 5.5 kg / year (Jan Kole et al., 2017). So far, there has been only a few studies that have detected tire microplastics in environmental samples. Research on this topic, especially on environmental samples, is insufficient.

This study attempted to identify microplastics from transport, mainly targeting microplastics from tyres (J. Worek et al. 2022). Fourier transform infrared (FTIR) spectroscopy was used for the identification of synthetic rubber, most likely derived from car tyres. To confirm the results from FTIR, the complementary method of molecular spectroscopy, namely Raman microspectroscopy, was applied. Soil samples and road dust from the areas with heavy traffic. An average of 372±50 fragments per kilogram of dry weight were detected in the soil samples. In the case of road samples, this number was 515±20 fragments per kilogram of dry matter. More than 90% of the black fragments later identified as synthetic rubber were found in the samples. The microplastics from the road dust was less degraded than the microplastics from the soil. The influence of the environment in which microplastics reside on the leaching of various substances, especially organic compounds, is still a big gap in knowledge. The great advantage of the research conducted is the combination of infrared and Raman methods. These are complementary methods.

#### 4.14.P-Th362 Deposition of Airborne Microplastic and Tire Wear Particles in Salt Marsh Habitats: Proximity to Bridges

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The pathways by which microplastic and tire wear particles (TWP) enter coastal ecosystems remain poorly understood, especially those pathways transporting airborne particles. As such, the objective of this study was to assess the deposition of airborne microplastics, including TWP, into three salt marsh and salt marsh-adjacent locations around Charleston Harbor, SC (USA). To accomplish this, we collected samples using stainless steel buckets (324 cm<sup>2</sup>) through passive deposition during both wet and dry periods. Retained particles were filtered onto glass fiber filters and visually analyzed under a dissecting microscope. Suspected microplastic particles were confirmed as synthetic with the hot needle test, and a subset were examined using FTIR spectroscopy. Microplastic deposition was dependent upon location ( $p < 0.0001$ ) with the highest abundances associated with the site adjacent to a bridge crossing Charleston Harbor ( $4,825.8 \pm 816.5$  MP/m<sup>2</sup>/day). Most particles (95.3%) at this site were TWP. Deposition at the other two sites ranged from 137-802 particles/m<sup>2</sup>/day and were not significantly different from one another. At these two sites, fibers and TWP were the most common particles. Deposition of airborne microplastics at all three sites was not dependent upon precipitation ( $p = 0.60$ ). Future research will investigate the morphological features of the particles (size and shape), and the correlation between particle deposition at these sites and meteorological factors (wind speed and direction). These results suggest that proximity to sources, such as roadways and bridges, significantly influence the abundance and types of microplastic particles deposited in salt marsh habitats through airborne pathways. This is the first evidence of airborne TWP in the size fraction 63 - 5000  $\mu\text{m}$  being deposited in salt marshes and underscores the need to include airborne pathways when holistically assessing microplastic exposures in these sensitive coastal habitats.

#### 4.14.P-Th363 Quantification of TWP/TRWP in PM10 Airborne Dust of Contrasting Sites

**Juanita Rausch, Particle Vision, Switzerland**

The link between particle pollution and health issues has been widely demonstrated during the last decades. Consequently, the WHO recommended new limit values for particulate matter PM10 and PM2.5 in September 2021. Following these recommendations it can be expected that many inhabited areas are affected by PM10 and PM2.5 exceedances. Therefore, authorities have more than ever the need of seeking lowering PM values. To achieve this by implementing targeted dust mitigation measures it is necessary to first obtain a better understanding on the fine dust constituents. Unfortunately, there is no one single method that can characterize the totality of PM10 owing to the intrinsic different characteristics of particles, and hence, stability and detectability with the various available methods. In particular, the differentiation and quantification of tyre/road wear particle mixtures (TWP/TRWP) has not been yet routinely included in the PM characterization and monitoring due to the analytical difficulties encountered, so far. Here, we present a newly developed approach based on morpho-chemical single-particle analysis by Scanning Electron Microscopy (SEM) coupled to Energy Dispersive X-ray Spectroscopy (EDX) complemented with a machine-learning (ML) based algorithm that classifies and quantifies TWP/TRWP in passively collected (Sigma-2, VDI 2119:2013) airborne dust samples.

Measurements at multiple sites with contrasting characteristics were performed in Switzerland (Bern, Zurich and Bussigny) and Great Britain (London). Clear differences in the concentration of TWP/TRWP were observed between the various site typologies with the highest TWP/TRWP concentrations in Switzerland at the traffic/urban canyon site in Bern ( $2.2 \mu\text{g}/\text{m}^3$  annual mean) and the lowest in the urban background site in Zurich ( $0.3 \mu\text{g}/\text{m}^3$  annual mean). Furthermore, seasonal variations most likely related to (a) meteorological conditions likely to induce particle accumulation and subsequent enhanced resuspension, and to (b) higher biogenic activity during autumn and summer were detected.

Similarly, among the London sites, the urban background site presented the lowest TWP/TRWP and metal wear concentrations (in average,  $0.5 \mu\text{g}/\text{m}^3$  and  $0.7 \mu\text{g}/\text{m}^3$ , respectively) resembling values determined in the urban background site in Zurich (CH). The traffic site with a speed limit of 50 mph showed the highest concentrations with  $2.0 \mu\text{g}/\text{m}^3$  of TWP/TRWP and  $9.5 \mu\text{g}/\text{m}^3$  of metal wear particles in average.

#### 4.14.P-Th364 Roadway to Linking Exposure and Effects of Highway Stormwater Runoff and Particulate Matter – First Case Study Results from a Highly Frequented Highway in Germany

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Awareness and knowledge about road runoff toxicity and tire and road wear particles (TRWPs) are rapidly increasing. Yet, the fate and ecotoxicological effects outside of communal wastewater treatment systems are insufficiently understood paired with a lack of data about how TRWPs interact with other road runoff pollutants.

Consequently, aquatic environmental risk assessment of TRWP polluted runoff faces the following challenges: **(1)** Due to structural and chemical variability of TRWP, no standardized sample preparation protocols for (bio-)chemical assessment are available; **(2)** The lack of environmentally relevant effect data complicates *a priori* decisions on endpoints of interest to investigate. Therefore, comprehensively investigating the ecotoxicity of TRWP demands a bottom-up scientific approach generating a broad knowledge base covering both chemical and biological effect information for different environmental model scenarios.

The project *RoadTox* aims for a quantitative ecotoxicological risk assessment of stormwater runoff sampled from highly frequented urban, country, and highway roads. Here we present the current project state with a special focus on results obtained

from an extended fish embryo toxicity assay battery on *Danio rerio* embryos combining several sublethal endpoints. Presented data includes standard sublethal effects complemented with spontaneous tail coiling, heartbeat-tracking, light-dark-transition responses and *in-vivo* EROD-activity. Additionally, supporting *in-vitro* data (e.g., EROD, estro- and androgenicity), chemical data, and road site biofilm community information from metabarcoding will add to the discussion. Current results strongly indicate that road runoff toxicity is largely driven by particulate-bound contamination. Applied sublethal non-standard endpoints in zebrafish (e.g., light-dark transition response and *in-vivo* EROD) were able to detect effects in low concentration ranges ( $\leq EC_{10}$ ). Chemical profiles strongly depend on preceding weather conditions but display a mutual composition pattern between different samples obtained from the same site. Both PAHs and metals concentrations in parts reached concentrations which may impact fish health. However, at this stage, no general conclusion is possible yet.

#### 4.14.P-Th365 Effects of Nano Tire Wear Particle Exposure and Increasing Temperature on an Estuarine Indicator Species

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Tire wear particles (TP) are synthetic rubber particles commonly released into the environment from automobiles. Our lab has previously found that the model organism, mysid shrimp (*Americamysis bahia*), exposed to nano-size TP showed changes in behavior and growth. Further, preliminary results from our lab indicate that temperature in combined exposures to nanofibers may also drive physiological changes in mysid shrimp. To provide insight into how organisms may fare with increasing TP pollution and changing ocean conditions, we will run a factorial experiment testing the effects of varying TP concentrations (5, 50, 500 particles/mL) and temperatures (12, 14, 16 C) on mysid shrimp. After a 7-day exposure, we will examine behavior, hypoxia tolerance, reactive oxygen species, and caloric value. The results of our experiments with model organism *A. bahia* will show multi-stressor effects of pollution and climate change and may indicate how populations of a mysid shrimp species off of the Oregon Coast, *Holmesimysis sculpta*, a significant component of gray whale diets in the same order, may be impacted by increasing marine debris and ocean temperatures.

#### 4.14.P-Th366 Chronic Effects Induced by End-of-Life Tire (ELT) Derived Rubbers: An Integrated Approach by Biomarkers and Proteomics

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Every year a large amount of tires reaches their end-of-life and becomes wastes. Most of the so-called End-of-life-Tires (ELTs) are recycled to produce other objects or used for energy recovery. The aim of this study was the evaluation of the chronic toxicity induced by leachates of ELT-derived rubber granules (GR; from 0.8 to 2.5 mm, rough size) and powder (PW; < 0.8 mm, rough size) in the freshwater model *Danio rerio* (zebrafish). We firstly investigated the leachate composition (100 mg/L) characterizing the released chemicals, as well as the eventual presence of micro- and nanoparticles. Subsequently, we exposed the zebrafish embryos from 0 to 120 hours post fertilization (hpf) to the ELT leachates. Considering the Lowest Observed Effects Concentration (LOEC) of 10 mg/L ELTs, obtained in our previous study on zebrafish, we decided to perform the exposure to 0.1, 1 and 10 mg/L of both GR and PW. As endpoints of sub-lethal effects, we performed some biomarkers of cellular stress, as the activity of the antioxidant/detoxifying enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and ethoxyresorufin-O-deethylase (EROD), as well as the measurement of Reactive Oxygen Species (ROS). As biomarkers of neurotoxicity, we evaluated the activity of acetylcholinesterase (AChE) and monoamine oxidase (MAO). Subsequently, also the high-throughput analysis by proteomics was performed to highlight the eventual modulation of zebrafish proteins. We observed only a significant modulation ( $p < 0.01$ ) of EROD activity in specimens exposed to 1 and 10 mg/L of GR, while proteomics revealed the modulation of many proteins, from 37 in the organisms exposed to 0.1 mg/L of GR to even 108 in both 0.1 and 1 mg/L of PW experimental groups. The network analysis highlighted that many modulated proteins are involved in nitrogen and aromatic compound metabolism. This evidence suggests the possible adverse effects induction by the chemicals in the ELTs, as polycyclic aromatic hydrocarbons (PAHs; presents in ELTs as carbon black contaminants) and nitrogen compounds (e.g. adsorbed during the road activity). Currently, based on the high release of Zn by ELTs, we are measuring the metallothionein induction. Lastly, as apical endpoints, we are performing the analysis of behavioural alteration, to also provide the mode of action of ELTs on freshwater biological model.

#### 4.14.P-Th367 Daphnia Reproductive Impacts Following Chronic Exposure to Micro and Nano-Scale Particles from Three Types of Rubber

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Environmental sampling has documented high levels of black rubber microplastics in aquatic environments. These are often attributed to particles shed from driven tires; however, the use of recycled, or crumbed, rubber particles in artificial turf and flooring is another source of these particles. Studies have shown that acute exposure to micro, and nano-sized tire particles, as well as tire leachate can be toxic to aquatic organisms. Chronic toxicity assessments with tires are limited, and there is a need to evaluate impacts of rubber particles in smaller size-classes and from different sources to better understand the role that these features play in conferring toxicity. We investigated the impact of chronic exposure of *Daphnia magna* to micro (1-20  $\mu\text{m}$ ,  $3.13 \times 10^4 - 1.25 \times 10^5$  particles/ml) and nano (<1  $\mu\text{m}$ ,  $1.25 \times 10^5 - 1.0 \times 10^7$  particles/ml) sized rubber particles. The rubbers included tire

particles (TP) and two types of rubber from recycled tires (called recycled rubber (RR) and crumb rubber (CR) based on their product labels). Triplicate exposures began at 7 days old and lasted for a total of 28 days. Mortality, reproduction, and molting were assessed daily, and growth was measured at the end of the exposure. Additionally, the F<sub>1</sub> generation was reared for 28 days in particle-free water to assess multigenerational impacts. Exposures were conducted at sublethal levels where mortality was not observed. Chronic exposure to the micro rubber particles had severe impacts, delaying, decreasing and even eliminating reproduction starting at  $6.25 \times 10^5$  particles/ml. Chronic exposure to the nano rubber particles had less severe impacts, but delayed and decreased reproduction at the highest exposure level,  $5.0 \times 10^7$  particles/ml. Exposure to nano rubber in the parental generation had impacts on reproduction in the F<sub>1</sub> generation. Compared to acute exposures, much lower concentrations of rubber particles in both micro and nano sizes caused impacts for chronically exposed *D. magna*. Furthermore, the immense reproductive effects that chronic exposure to rubber particles have shown could have devastating impacts on populations of aquatic invertebrates.

#### 4.14.P-Th368 Toxicity of Tire Particles From Personal Mobilities on Soil and Aquatic Organisms

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Tire particles (TPs) generated from the roads are one of the microplastic source in the ecosystem. These particles directly release to soil and contact with water-media and leaching some additive-chemicals. TPs are continuously exposed to both soil and aquatic environments and threaten the living organisms. Using the TPs and TPs-leachate, the overall toxicity of TPs was evaluated. Three types of TPs (from bicycle, car, and electric scooter) were prepared and then exposed to *Vigna radiata* and *Folsomia candida* at 10 g/kg soil concentration. TPs-leachates were also prepared and then 50% concentration of leachate was exposed to aquatic organisms (*Daphnia magna* and *Danio rerio*). TPs directly induced the inhibition on the growth of *V. radiata* and *F. candida*. TPs-leachate also disturbed the movement of *D. magna* and normal development on *D. rerio*. This result suggests that TPs from road directly induce the growth inhibition on soil organism and release some chemicals, eventually affecting aquatic organisms.

#### 4.14.P-Th369 Developmental Toxicity of Recycled Tire Wear Microplastic Leachates to Larval Lamprey (*Lampetra planeri*)

**Flora Rendell-Bhatti<sup>1</sup>**, Arne Jacobs<sup>2</sup>, Elma Lahive<sup>3</sup>, Eva Jimenez-Guri<sup>4</sup> and Colin Bull<sup>5</sup>, (1)Biological and Environmental Sciences, University of Stirling, United Kingdom, (2)University of Glasgow, United Kingdom, (3)UK Centre for Ecology & Hydrology (UKCEH), United Kingdom, (4)University of Exeter, United Kingdom, (5)University of Stirling, United Kingdom

Whilst research on the occurrence of microplastic in freshwater environments has been increasing in recent years, the consequences of the presence of microplastics for these ecosystems are not elucidated. Particularly, whether the early developmental stages of protected vulnerable species are impacted is a pertinent question when trying to understand the consequences of plastics in our natural environment. Untreated road run-off is a significant source of tyre wear particles and associated contaminants to the freshwater environment, often exceeding the lethal concentration of some chemicals to aquatic biota. The impacts of tyre wear particle contamination on early developmental life stages are understudied, and yet the outcomes of this sensitive period being disrupted could have important ecological consequences. Tyre wear particles are a substantial proportion of microplastics reported in the aquatic environment and they can leach a range of chemical compounds, which have been shown to elicit toxic effects. Lamprey species in Europe are undergoing important population declines due to a multitude of stressors, which are essential to understand if we are to conserve and manage threatened populations. To the best of our knowledge, this is the first study to observe developmental defects that leachates from tyre wear particles elicit in lamprey embryos. We use recycled tyre wear particles to generate a microplastic leachate in which embryos were cultured. Preliminary data suggest that exposure to leachates, following 10-day incubation of  $<65\mu\text{m}$  tyre wear particles at 0.5%, elicits consistent defects in the brook lamprey (*Lampetra planeri*) embryos. The phenotype we observe in the microplastic treatment suggests that compounds in the leachate weaken the vitelline membrane, causing the yolk to leak out. This study documents developmental defects in larval lamprey which were exposed to leachates of tyre wear particles, demonstrating the potential contribution of this stressor to the population status of this vulnerable species. Further research on the ecotoxicological impacts of tyre wear particles is needed to aid our understanding of whether this complex contaminate has a role in lamprey population declines.

#### 4.14.P-Th370 Gene Expression Analysis of *Chironomus riparius* in Response to Acute Exposure to Tire Rubber Particles and Leachates

**Patricia Caballero Carretero<sup>1</sup>**, Victor Carrasco Navarro<sup>2</sup>, Jussi Kukkonen<sup>2</sup> and Jose-Luis Martinez-Guitarte<sup>3</sup>, (1)National Distance Education University (UNED), Spain, (2)University of Eastern Finland, Finland, (3)UNED, Spain

Aquatic pollution caused by tire rubber microparticles (TRP) entering aquatic ecosystems through stormwater is an important challenge. Tire rubber includes many microplastic particles and chemical additives which are a cause for concern. Currently, the information on the molecular effects of TRP, or specially its additives, in freshwater organisms is scarce.

To address this problem, an array covering different cellular processes has been designed for the freshwater midge *Chironomus riparius*. Fourth-instar larvae were exposed to two concentrations of TRP ( $1 \text{ mg} \cdot \text{L}^{-1}$ ,  $10 \text{ mg} \cdot \text{L}^{-1}$ ) and two concentrations of tire rubber leachates (TRL) (0.0125 %, 5 %) to evaluate the transcriptional activity by Real-Time PCR. To assess acute toxicity, larvae were exposed for 24h and genes related to endocrine system, stress response, DNA repairing mechanisms, oxidative stress, and detoxification mechanisms were evaluated. The activity of the enzymes: glutathione S-transferase (GST) and catalase was also examined.

The main pathway affected was the stress response, showing overexpression of HSPs. Moreover, there is a reduction of the *GSTd3* and *catalase* disrupting the antioxidant system. Most of these alterations are caused by TRL, showing higher toxicity than TRP. The results obtained in this work provide the first approach at the molecular and cellular levels to elucidate the impact of

TRL in freshwater organisms. To perform a realistic evaluation of the TR effects, additional research is required to assess the TR long-term effects at the molecular level.

#### **4.14.P-Th371 Mitigation of Tire Rubber Microplastic Ecotoxicity by Combined Vacuum UV and UV-C treatment**

*Emil Bock Nielsen<sup>1</sup>, Niels Iversen<sup>1</sup> and Peter Roslev<sup>2</sup>, (1)Department of Chemistry and Bioscience, Aalborg University, Denmark, (2)Aalborg University, Denmark*

Synthetic rubber is one of the most widely used plastic polymers in products such as footwear, rubber bands and vehicle tires. A significant source of microplastics in the environment is tire rubber microplastics (TRMP) and the average global emission of TRMP has been estimated to 0.8 kg/year per capita. Some TRMP settle close to the emission source, but a significant fraction can be transported in air and water between environmental compartments, and may eventually end up in surface waters. Degradation of TRMP in surface waters is very slow and can take hundreds of years. Most TRMP are therefore persistent pollutants in aquatic ecosystems. The effects of TRMP on most aquatic life is poorly understood but significant ecotoxicity to some species has been observed. A potential degradation pathway for TRMP is photodegradation and in particular exposure to short-wave ultraviolet light (UV). This study investigates the effects of short-wave UV irradiation of TRMPs and TRMP leachates on degradation and ecotoxicity. TRMP particles and leachates in water were exposed to different UV regimes including UVC (254 nm) and combined UVC and vacuum UV irradiation (185 nm). Combined VUV/UVC irradiation has a potential to degrade TRMPs due to generation of different reactive species that can interact with rubber constituents. UV irradiation was conducted using a collimated beam setup with different UV exposure doses. Ecotoxicity of non-treated and UV-treated TRMPs was examined using a battery of test organisms that included the luminescent bacterium *A. fischeri*, the freshwater microalga *R. subcapitata*, and the crustacean *D. magna*. Changes in genotoxicity was measured using the SOS Chromotest. The study confirmed the presence of several toxic constituents in TRMP leachates including zinc and PAHs such as fluoranthene, pyrene and benzo(a)pyrene. Interestingly, VUV/UVC treatment removed toxic compounds from TRMP leachates and decreased the overall ecotoxicity. PAHs were degraded by up to 90% by VUV/UVC treatment and the apparent ecotoxicity decreased 5-fold as indicated by changes in median effective concentrations (EC50). These findings indicate a potential of VUV/UVC treatment as a method to mitigate toxic compounds in TRMP leachates.

#### **4.14.P-Th372 Investigation of in-vivo Biomarker Responses in *Danio rerio* Embryos Exposed to Highway Stormwater Runoff following different Precipitation Scenarios**

*Alexander Maria Pape<sup>1</sup>, Selina Seibold<sup>1</sup>, Markus Thomas Schmitz<sup>1</sup>, Kun Qiao<sup>1</sup>, Martin Krauss<sup>2</sup>, Regina Dolny<sup>3</sup>, Simone Lechthaler<sup>3</sup>, Volker Linnemann<sup>3</sup>, Henner Hollert<sup>1</sup> and Sabrina Schiwyl<sup>1</sup>, (1)Evolutionary Ecology & Environmental Toxicology, Goethe University Frankfurt, Germany, (2)Department of Effect-Directed Analysis, Helmholtz Center for Environmental Research, Germany, (3)Institute of Environmental Engineering, RWTH Aachen University, Germany*

In recent years, the adverse effects of road wastewater and tire and road wear particles (TRWP) have attracted increased focus in environmental sciences. However, distribution and ecotoxicological implications in the environment are poorly understood, and only few data on the interaction of TRWP and other road-related pollutants are available.

This study is part of the project "RoadTox: Ecotoxicological Assessment of Tire and Road Wear Particles in Stormwater Runoff of Heavily Frequented Roads", investigating the acute toxicity of highway runoff samples to aquatic organisms via exposure to sublethal concentrations of road runoff, including selected biomarkers.

Runoff samples were taken at the federal highway 4 in Aachen, North-Rhine Westphalia, Germany, during rain events. Samples were filtered and fractioned into three groups (unfiltered runoff, filtered < 63 µm, or 0.3 - 0.6 µm). This poster presents experiments with *Danio rerio* embryos assessing acute toxicity until 120 hpf according to FET DIN EN ISO 15088, as well as several *in-vivo* biomarker responses. Biomarker endpoints comprise dioxin-like activity (EROD), synaptic signalling (AChE) effects, as well as possible oxidative stress (e.g., glutathione peroxidase or catalase). In addition, this study compares several environmentally relevant scenarios throughout the year, e.g., snow melting events in spring or heavy rains after long dry periods, possibly leading to an increase in particles and pollutants in runoff and subsequent leaching of contaminant loads. Preliminary results show a strong difference in acute toxicity over the year and an increase in EROD activity, indicating metabolism and potential bioactivation of xenobiotics.

Native samples were chemically analysed for carbohydrates (PAH and mineral oils), (heavy) metals, and microplastics shared by the Institute for Environmental Engineering at the RWTH Aachen (ISA). For completion of data, organic extracts were analysed by the Helmholtz Centre for Environmental Research for a wide variety of common TRWP contaminants.

#### **4.14.P-Th373 Sub-cellular Effects of Tire Particles and Bioaccumulation of Their Associated Chemicals in the Aquatic Insect *Chironomus riparius***

*Rébecca Beauvais<sup>1</sup>, Thibault Masset<sup>2</sup>, William Dudefoi<sup>3</sup>, Kristin Schirmer<sup>3</sup>, Alan J Bergmann<sup>1</sup>, Etienne Vermeirssen<sup>1</sup>, Florian Breider<sup>2</sup> and Benoit J.D. Ferrari<sup>4</sup>, (1)Ecotox Centre, Switzerland, (2)Ecole Polytechnique Fédérale de Lausanne – EPFL, Switzerland, (3)Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (4)Centre Ecotox, Switzerland*

Through road runoff, tire and road wear particles (TRWP) can reach freshwater ecosystems and settle down in river or lake sediment. Benthic organisms, for instance insect larvae, will potentially be exposed to TRWP and associated chemicals (e.g. PAHs, metals, antioxidants/antiozonants) through direct contact and/or ingestion. While many ecotoxicological studies have been published on pelagic organisms, research concerning organisms living in or on sediment are scarce. Among them, chironomid larvae are a source of food for fish and therefore relevant to the trophic transfer of chemicals in aquatic ecosystems. To assess the potential effects of tire particles, we evaluated biological endpoints and bioaccumulation of TRWP-associated chemicals in

*Chironomus riparius*. For our experiment, we used cryogenically milled tire tread (CMTT) particles as a proxy for TRWP. We exposed 4<sup>th</sup> stage larvae for up to 4 days to clean sand or sand spiked with 0.5% CMTT (dw). The expression of 16 genes involved in detoxification, immunity, response to oxidative stress and endocrine response were assessed after the 4-day exposure. Bioaccumulation of CMTT-related chemicals were measured after 2, 3 and 4 days on depurated larvae. We observed no mortality nor effect on growth in larvae exposed to CMTT. We however observed a differential gene expression for 4 biomarkers, namely for a gene involved in immunity (*defensin*) and for the detoxification (*CYP450*). The dysregulation of this expression helped larvae to cope with the stress caused by the exposure to the spiked sand. Bioaccumulation kinetics showed chemical-dependant rates as some compounds reached steady state after 4 days of exposure while others did not. Overall, for most tire-associated chemicals, Biota-Sediment Accumulation factors were low and ranged from 1.3 to 6.3. Although the chironomids have accumulated CMTT-related chemicals, there was no ecotoxicological effects on 4<sup>th</sup> stage larvae at the individual scale. As such, contaminated larvae could represent an important source of TRWP-related chemicals for fish. Our study additionally helped to gain mechanistic knowledge of this particular mixture on model invertebrates. Future experiments will aim at studying the contribution of ingested particles to the accumulation of CMTT-associated chemicals in larval tissues. To support the molecular responses, the level of activity of enzymes, (e.g. phenoloxidase for immune response, catalase for oxidative stress) will be analysed.

## Track 5: Life Cycle Assessment and Foot-Printing

### 5.01 Circularity, Recycling and Multi-Functional Systems

#### 5.01.P-Mo342 Applying System Expansion with Multiple Functions to Address the Overall System Burdens of Recycling in Comparison to other Circular Economic Material Strategies

*Simon Alexander Saxegård, Fredrik Wikström and Helén Williams, Karlstad University, Norway*

It is common in LCA today to environmentally evaluate material recycling from a product level perspective. A problem with the product level perspective is that the total burden of recycling is divided into several product value chain stages that are inherently connected by the recycled materials without fully disclosing the overall system level burden of recycling. Therefore, it is challenging to compare recycling to otherwise linear not-circular product material strategies and derive at one unambiguous conclusion at the overall system level, and in turn the societal level perspective. Amplifying the multi-result output problem when addressing the different stages of recycling from the product level perspective is that there exist many LCA methods for dividing the burdens across the three product life cycles stages connected by the recycled material of which many give different results for the same material recycling scenario. Our suggestion to solve for both of these problems is to address recycling at the overall system level using the LCA method of System Expansion with Multiple Functions (SEMF) by summing together recycle-cascading product life cycles using methods that maintain physical realism at the overall system level and compare these to the alternative Circular Economic (CE) material strategies Reduce, Replace, Recover or any combination of these for an equal number of products. To ensure physical realism, we employ MFA to trace the flow of recyclable and not-recyclable materials through the individual product value chains and across the recycle-cascading product value chains. Our hypothesis is that once we can find one overall system level environmental impact result for recycling, we can compare recycling to and in combination with other material strategies to determine its environmental performance with basis in regionalized socio-technological conditions. The main results shall demonstrate whether the MFA based SEMF method enables a compatible comparison of recycling to other material strategies at the overall system level, and the underlying differences between the product level and overall system level LCA assessment method scopes. This study provides a suggestive LCA framework for addressing the long-standing conundrum in LCA regarding how the academic society can environmentally evaluate recycling relative to other material strategies and gain one unambiguous finding at the overall system level.

#### 5.01.P-Mo351 Contextualized Phosphorus Recycling: Potential Diminution of Phosphorus Criticality at Territory Scale - Application to Agricultural LCA

*Perlette Totoson, INRAE, France*

Phosphate rock is classified as critical raw material for the European Union's economy. It provides phosphorus (P), which is an essential element for food production and is used as a mineral fertilizer. Due to a lack of a phosphorus substitute in fertilization, recycling is presently the only solution to reduce criticality, especially for countries such as France, that has no phosphate rock reserve and thus, must depend on foreign countries for its P needs for agricultural purposes. MAFOR or fertilizer mineral/organic matter from waste can contain available phosphorus. However, the recycling of P from these deposits for agriculture involves many actors (farmers, regulators, etc.) and depends on the studied spatial scale and its context (economic, regulatory, social, etc.) which can be either constraints or drivers for P recycling. Nevertheless, these issues are not taken into account in the recycling rate used in the raw material criticality assessment nor in the LCA methods. Therefore, it is necessary to contextualize P recycling for a better understanding of phosphorus recycling, and to allow a relevant phosphorus criticality assessment and an environmental impact assessment of phosphorus recycling at a local scale. In order to achieve this, a contextualized model is developed. The goal is to be able to quantify phosphorus which would potentially be used by farmers, originated from the total available quantities of deposits, by respecting the context of the studied territory. The originality of the model lies in the integration of context-factors that can affect the use of P contained in MAFOR by farmers. The model will be applied in two territories in France, which are different regarding the origin of MAFOR produced. As output of the model, four indicators are expected: the effectiveness and efficiency of phosphorus recycling, the dependence on phosphate mineral fertilizers imported from outside France and the contextualized balance between supply and demand in P recycling-derived fertilizers. The model

gives a methodology advancement, which would improve or be a complement of the LCA tool to assess territorialized recycling scenarios. The output indicators would improve the understanding of P circularity and, help determine a suitable P recycling pathway to reduce P criticality at a territorial scale and relevant from an environmental point of view.

#### **5.01.P-Mo353 Life Cycle Assessment of emerging Carbon Capture for Utilisation Technologies: Methodological Archetypes at VIVALDI Project**

**Jorge Senan-Salinas**, Sergio Ponsá, Laia Llenas and Joan Colon, *University of Vic-Central University of Catalonia, BETA Technological Center, Spain*

Carbon Capture technologies for Utilisation (CCU) aim to mitigate climate change through the sequestration of CO<sub>2</sub> from industrial streams within the structure of valuable products for the Technosphere (such as chemicals). Therefore, understanding the environmental impacts of the technology at early stages is useful to reach the final aim of the research. For this purpose, Life Cycle Assessment (LCA) has been pointed out as the most suitable tool. Nonetheless, this methodology has relevant methodological choices that could affect the final decision. Some authors distinguish these issues between non-CCU-specific and CCU-specific.

Among the non-CCU specific issues, we found common LCA issues such as the definition of the functional unit, the approach, the allocation method and so on. Nonetheless, there is an apparent emerging consensus in the definition of methodological choices through the specific definition of the goal. This goal should define, for instance, if the assessment is oriented to understand the process or the product impact (Process-oriented versus product-oriented), if the main goal is to account for the impact or understand the consequent impacts of a decision (attributional versus consequential approach).

On the other hand, besides the CCU-specific issues, a few points should be remarked such as the consideration of the CO<sub>2</sub> streams (as a co-product or waste), the source (fossil or biogenic), the legal status, the business model of the CCU or the policy framework. This discussion can have some relevance in the methodological choices and can interfere with the abovementioned frameworks.

VIVALDI project aims to convert biogenic carbon dioxide from bioindustries into CO<sub>2</sub>-based organic acids. The poster will summarise the main potential archetypes identified (n>10) in the context of the VIVALDI project and how these methodological archetypes can be correlated with specific goals and interact with contexts such as the business models, type of CO<sub>2</sub> sources, and potential policy frameworks of the near future.

#### **5.01.T-01 Environmental Assessment of Producing Rigid Polyurethane Foams Using Unrefined Crude Glycerol - Different Strategies**

**Paula Quinteiro**<sup>1</sup>, Nuno V. Gama<sup>2</sup>, Artur Ferreira<sup>2</sup>, Ana Dias<sup>2</sup> and Ana Barros-Timmons<sup>2</sup>, *(1)CESAM, University of Aveiro, Portugal, (2)University of Aveiro, Portugal*

Rigid polyurethane foams (PUFs) are normally used in thermal insulation for construction mainly because of its thermal insulating and mechanical properties. These foams are synthesized by reacting a polyol with an isocyanate in the presence of a catalyst, a surfactant and a blowing agent. So far, most of the polyols used are derived from crude oil feedstocks. Yet, the increasing concerns over environmental issues have been promoting the use of renewable/recycled feedstocks, such as crude glycerol (CG) derived from bio-based feedstocks, like soybean, rapeseed, palm and waste cooking oil (WCO). In turn, from the perspective of a circular economy, recycling PUF waste to recover its polyol or CG content and use it as a partial substituent of virgin polyol or CG in the production of new rigid PUFs (close-loop recovery), rather than disposing it in landfill, is generally seen as a suitable option.

To evaluate that perception, this study compares the environmental impacts of rigid PUF produced using polyol derived from crude oil and unrefined CG derived from bio-based feedstocks, based on life cycle assessment following a cradle-to-grave approach. Three optimised formulations of PUF derived from polyol/CG were considered. Furthermore, recycling of rigid PUF wastes, to recover its polyol/CG, was compared with its disposal in landfill. The results obtained revealed that the different formulations used in this study had distinct impacts depending on the scenarios considered. Moreover, these results have also clearly demonstrated that, overall, the environmental superiority of bio-based feedstocks compared to their fossil feedstock counterpart to produce rigid PUFs cannot always be claimed. Regardless of the scenario considered, the methylene diphenyl diisocyanate is the main hotspot for all impact categories other than marine eutrophication (ME), ranging from 50 to 98% of the total impacts. As regards the scenarios involving polyol/CG recovery, the environmental impacts from the polyol/CG recovery process exceeded the environmental benefits from the PUF waste recycling and the inherent partial replacement of virgin polyol/CG. Indeed, the polyol/CG recovery resulted in a slight increase of the total impacts compared to those without polyol/CG recovery. The perception that introducing the concept of circularity in the rigid PUF production leads to better environmental performance of this industry will require significant improvements in the chemical recycling of PUF.

#### **5.01.T-02 Key Challenges of Bio-fertilisers Modelling (LCA And Non-lca Indicators): A Roadmap to a Consensual Pef-compliant Methodology in the Circular Economy Transition of Agricultural Systems**

**Jorge Senan-Salinas**<sup>1</sup>, Nancy Peña<sup>1</sup>, Karetta Timonen<sup>2</sup>, Pasi Korkalo<sup>2</sup>, Daniel Egas<sup>1</sup>, Laia Llenas<sup>1</sup>, Juha-Matti Katajajuuri<sup>2</sup>, Joan Colon<sup>1</sup>, Ilkka Leinonen<sup>2</sup>, Marzena Smol<sup>3</sup> and Paulina Marcinek<sup>3</sup>, *(1)University of Vic-Central University of Catalonia, BETA Technological Center, Spain, (2)Natural Resources Institute Finland (Luke), Finland, (3)Mineral and Energy Economy Research Institute of the Polish Academy of Sciences*

Nutrient-recovering technologies and alternative fertilisers are key elements in the Circular Economy transition of societies and the agro-food industry, in particular. They are embedded within European policies such as *Farm-to-fork* and new Fertilising Product regulations. These products and technologies allow the obtention of valuable nutrients such as nitrogen (N), phosphorous

(P) or potassium (K) from different waste sources such as organic wastes from agricultural and livestock sectors or urban and industrial wastewater.

Life Cycle Assessment (LCA) has been pointed out as a useful tool for holistic environmental assessment. Generic ISO standards (eg. ISO 14040/4, ISO 14067) and different guidelines, including the Product Environmental Footprint (PEF) guide. Nonetheless, they leave numerous open questions and choices of LCA practitioners which might easily direct to the situation where different LCA studies with different choices are not at all comparable. This lack of consensus is an important barrier to benchmarking fertiliser products and delays the decision-making of policymakers, industrial stakeholders and consumers. Moreover, several sustainability issues are only partially covered by LCA impact categories; for example, alternative fertilisers can impact biodiversity, soil properties and soil carbon sink capacity as well as have an effect on ecosystem services. The scientific consensus on what are the main environmental issues and how to assess them is not well covered and is needed.

NOVAFERT project (EU-funded Horizon Europe grant agreement No 101060835) aims to propose a starting point to fulfil this normative gap by proposing a PEF-compliant methodology of alternative fertilising products covering every life cycle stage: production, storage, distribution and application.

The general methodology follows these three steps: i) the mapping of the current state-of-the-art and identification of key challenges (e.g. emissions modelling, non-LCA indicators) and main methodological choices (e.g., functional unit, system boundaries) of the alternative fertilisers modelling within the existing guidelines, databases (Ecoinvent, Agribalyse...), ii) the assessment of the adaptability of these challenges to PEF normative constraints and its potential to generate a broader consensus; and iii) the establishment of a public debate to route towards a consensual method (e.g., fertiliser scientist, soil scientists, LCA community, industry).

#### **5.01.T-03 Comparative Environmental Life Cycle Assessment of Agrivoltaic Systems in Austria Using a System Expansion Approach**

*Theresa Krexner, Alexander Bauer, Francisco Medel, Andreas Gronauer and Iris Kral, Institute of Agricultural Engineering, University of Natural Resources and Life Sciences, Austria*

Agrivoltaic (APV), the double use of agricultural land for food/feed and electricity production via photovoltaic (PV)-modules, has recently been a popular topic in research. So far, no holistic assessment of environmental impacts on an agricultural site in Austria has been conducted. Therefore, this study compares a tilted (S-) with a vertical bifacial (VB-)APV system and further the simple management scenarios of an unmodified agricultural production (Agri-only) and an open-space PV system (PV-only) by using life cycle assessment. To conduct a fair comparison of mono-use of land (Agri-only and PV-only) with a dual-use (APV scenarios) on a comparable basis, every scenario needs to provide the same outputs. Based on ISO 14044 recommendations to deal with multi-outputs, the methodical approach of system expansion is applied with a common functional unit (FU) above all scenarios. Hence, in Agri-only an additional electricity production (Austrian production mix or green electricity) is added, while agricultural production is added in PV-only. As FU a sum of outputs is used: 1 kWh electricity+60 g of agricultural goods. The impact categories global warming potential (GWP), human toxicity (HCT), terrestrial ecotoxicity and acidification (TAP), freshwater and marine eutrophication (MEP), fine particulate matter formation, mineral and fossil resource scarcity are assessed. Results show that Agri-only with Austrian produced electricity leads to the highest environmental impacts in four assessed impact categories, S-APV in three categories. Overall, VB-APV can reduce environmental impacts by 15-81% compared to the Agri-only scenario with Austrian produced electricity, S-APV by 3-70% except for HCT and TAP, where the scenario has the highest contributions due to high steel demand for the mounting structure and the PV-module production. A hotspot in both APV-scenarios is the PV-module production in China, due to the high demand and impact of electricity, in the S-APV scenario further the steel mounting structure. Results show a GWP of 49 g CO<sub>2</sub> eq. per FU for VB-APV and 73 for S-APV, respectively, while being slightly higher for PV-only (80 g CO<sub>2</sub> eq. per FU); for the Agri-only scenario it depends which electricity mix is used for system expansion (26 and 176 g CO<sub>2</sub> eq. per FU for the green and Austrian mix, respectively). Overall, it is shown that APV-systems can reduce environmental impacts compared to the Agri-only scenario with Austrian produced electricity.

#### **5.01.T-04 Exploring Life Cycle Assessment Methodologies for Multi-Functionality in the Steel Sector with a CCU Example**

*Marta Cruz Fernandez<sup>1</sup>, Peter Hodgson<sup>1</sup> and Jon McKechnie<sup>2</sup>, (1)Tata Steel UK, United Kingdom, (2)Faculty of Engineering, University of Nottingham, United Kingdom*

An ever-growing body of scientific evidence proves climate change and its dangerous effects. To tackle this, the focus has been on stopping the increase of greenhouse gas (GHG) emissions as soon as possible, with the long-term goal of achieving a near zero emissions by mid-century. The iron and steel industry is a major emitter of greenhouse gases (GHG), responsible for 7-9 % of global anthropogenic CO<sub>2</sub> emissions. Some of the alternatives to reduce emissions include the use of Carbon Capture Utilisation and Storage (CCUS) and new iron and steelmaking processes.

The use of CO<sub>2</sub> as a carbon source allows the synthesis of valuable products with a lower carbon footprint than those produced from fossil fuels. The latest projections by Volker et al. demonstrate a large potential for CO<sub>2</sub> capture and utilization by 2050. At 10% market share, CCU could permanently remove over a gigaton of atmospheric CO<sub>2</sub> annually, and, as a carbon feedstock for the circular economy, could replace even more new fossil carbon.

Overall, CCU has key advantages relative to CCS: it provides a means to create valuable carbon-based products, and it can be instrumental in promoting a circular carbon economy. To ensure its effectiveness, CCU must be rapidly scaled to a global industrial level while being thoughtfully planned and consistently evaluated to minimize unintended consequences. This emphasizes the need for robust methods to assess the environmental impacts of the CO<sub>2</sub>-based products. Life Cycle Assessment



(LCA) is a standardised method to evaluate the environmental impacts of processes and products by including all life cycle steps from the cradle to the grave.

The application of LCA to CCU can help support decision making and prioritizing different CCU technologies and products. However, there are different issues in dealing with CCUS within LCA. Goal and scope definition. It is possible to set up the LCA study from two different perspectives, the CO<sub>2</sub> producer or the CO<sub>2</sub> user. This will impact the system boundaries and functional unit. Allocation methods for CO<sub>2</sub>-based co-products. An important methodological choice in Life Cycle Assessment (LCA) lies in the question of how to deal with multifunctional systems (for example, the production steel and a CO<sub>2</sub>-based fuel).

This presentation will discuss the issues of attributing emissions and environmental impacts to steel industry products and co-products in LCA studies, through the example of CO<sub>2</sub>-based fuels.

## 5.01.P Circularity, Recycling and Multi-Functional Systems

### 5.01.P-Mo342 Applying System Expansion with Multiple Functions to address the overall system burdens of recycling in comparison to other Circular Economic material strategies

*Simon Alexander Saxegård, Fredrik Wikström and Helén Williams, Karlstad University, Norway*

It is common in LCA today to environmentally evaluate material recycling from a product level perspective. A problem with the product level perspective is that the total burden of recycling is divided into several product value chain stages that are inherently connected by the recycled materials without fully disclosing the overall system level burden of recycling. Therefore, it is challenging to compare recycling to otherwise linear not-circular product material strategies and derive at one unambiguous conclusion at the overall system level, and in turn the societal level perspective. Amplifying the multi-result output problem when addressing the different stages of recycling from the product level perspective is that there exist many LCA methods for dividing the burdens across the three product life cycles stages connected by the recycled material of which many give different results for the same material recycling scenario. Our suggestion to solve for both of these problems is to address recycling at the overall system level using the LCA method of System Expansion with Multiple Functions (SEMF) by summing together recycle-cascading product life cycles using methods that maintain physical realism at the overall system level and compare these to the alternative Circular Economic (CE) material strategies Reduce, Replace, Recover or any combination of these for an equal number of products. To ensure physical realism, we employ MFA to trace the flow of recyclable and not-recyclable materials through the individual product value chains and across the recycle-cascading product value chains. Our hypothesis is that once we can find one overall system level environmental impact result for recycling, we can compare recycling to and in combination with other material strategies to determine its environmental performance with basis in regionalized socio-technological conditions. The main results shall demonstrate whether the MFA based SEMF method enables a compatible comparison of recycling to other material strategies at the overall system level, and the underlying differences between the product level and overall system level LCA assessment method scopes. This study provides a suggestive LCA framework for addressing the long-standing conundrum in LCA regarding how the academic society can environmentally evaluate recycling relative to other material strategies and gain one unambiguous finding at the overall system level.

### 5.01.P-Mo343 LCA of Hybrid Smelting Process for Copper

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Renewable energy and electrification of industry will be critical in the journey to carbon neutrality. Copper is an essential resource in electric infrastructure and, thus will be critical on the journey to Net Zero. On the other hand, demand for copper is expected to increase, while supply is projected to fall short of forecast demand. For this reason, it will be necessary to sustainably expand the use of both copper ore and recycled raw materials to meet increasing demand.

This study analyzed data from JX Nippon Mining & Metals, which is actually engaged in copper mining development, copper smelting, and copper recycling, and conducted a LAC analysis based on various assumptions.

The Company aims to move in the following directions: (1) Reduce carbon footprint. (2) Increase recycling ratio (From 12% now to 50% in 2050). (3) Promote responsible procurement and ESG initiatives. (4) Form Green Enabling Partnerships.

CFT and other environmental impact assessments in JX Metals' trials suggested that the integration of ore-derived and recycled copper is a sustainable and stable supply system for copper from a holistic perspective.

The combination of primary resources and recycling will be the only possible solution for the sustainable copper supply. GHG emissions will increase by combustion of plastics adhering to end-of-life copper products. Development of the technology to remove plastics clearly from the scraps. Product design to facilitate separation of copper from other materials after disposal. Further GHG emissions reductions will be required for ore mining, beneficiation and transportation process. Introduction of renewable energy and electrification in mines and transport. Improvements in transport efficiency of raw materials.

To meet increasing demand for copper in future, it is required to ensure the sustainable copper supply by increasing the proportion of recycled raw materials to the primary smelting process.

Compared to steel, LCA analysis of copper lacks examples of initiatives, such as the absence of rules in ISO. It is necessary to reach a common understanding of LCA analysis methods for stable copper supply through many studies, including this study.

### 5.01.P-Mo344 Life Cycle Assessment of Steel Ladle Refractories Management According to Circular Economy Criteria

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We present the outcome of applying LCA to the management of ladle refractories by a steel factory in Spain, melting 1 million tonnes of steel scrap per year. Ladles are the vessels used to refine, transport and pour out molten metal, and constitute one of the pieces of equipment that consumes the largest amounts of refractories in a steel shop. The study addresses two situations, namely

before and after implementing circular economy strategies developed within the RFCS-funded project E-CO-LadleBrick. Such strategies include reduction of refractory waste generated in the ladles by optimising the ladle life, increasing amounts of worn bricks that can be reused in the production process or for other purposes, and increasing the amounts of waste refractories that can be recovered as secondary raw materials through recycling. The LCA applies consequential inventory modelling principles (multifunctionality dealt with by substitution, constrained suppliers excluded) and includes the entire life cycle of refractory products: mining of minerals, processing to obtain refractory raw materials, manufacturing of finished products, use phase and management as waste). Inventories were built for production of 12 different raw materials and 13 finished shaped and unshaped products. Data were also collected from the steel manufacturer in terms of refractories consumption, waste collection and treatment. As background database, the EXIOBASE v3 was used. The advantage of using an input-output based database as EXIOBASE is its high level of completeness, as well as the ability to cover processes in different countries with country-specific data. The main limitation of such a database, namely the level of aggregation of activities, is managed by disaggregating them whenever necessary, using process-specific data from other sources. The LCA addressed a set of 14 environmental indicators by means of the Stepwise method. The results show that adopting the developed strategies by this factory leads to annual savings of, among others, 2,000 tonnes CO<sub>2</sub>-eq/year. This is achieved mainly by improvements in waste management (reuse, remanufacture and recycling), leading to the substitution of primary products, and second by the reduction in refractories use as a result of optimising their useful lives in the ladles. A life cycle costing (LCC) study carried out in parallel to the LCA also showed substantial benefits, namely a reduction in life cycle costs by approximately 1 million €/year.

#### **5.01.P-Mo346 LCA as a Tool for Eco-design on Early Stages of Photovoltaic Technology Development: A Case Study on Silicon Heterojunction Tunnel – Interdigitated Back Contact (SHJ-IBC) Technology**

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The development of photovoltaic technology (PV) has allowed to convert sunlight into electrical energy and has made solar PV a fast evolving industry in the last decades. To reach climate targets, the decarbonization of the energy system is essential and for this, photovoltaic solar energy is expected to play a key role.

There exist several PV technologies under development. Silicon heterojunction tunnel – interdigitated back contact (SHJ-IBC) technology is promising in terms of efficiency and it is proven on lab-scale. Its scalability will be developed, with the goal of adding 30% to the total capacity of PV cell production in Europe (compared to 2020). Full control over the PV supply chain will not only smoothen the path for energy transition in Europe but it will also facilitate the knowledge about the environmental hotspots of this technology, so actions can be taken to lessen its impacts.

At this point, supporting the technological development from an environmental point of view is crucial to ensure low carbon footprint as well as enhance circularity. Eco-design at early stage of research development processes allows more sustainability improvements. Hence, Life Cycle Assessment (LCA) is used to support the eco-design of tunnel IBC PV modules and manufacturing lines towards zero-waste as well as comparing different end-of-life alternatives. This will also contribute to develop optimization roadmaps for potential recyclability.

Methodology Guidelines on LCA of PV elaborated by the International Energy Agency (IEA) and Environmental Footprint 3.1 impact assessment method will be used. The model will be done in SimaPro 9.4.0.2. Primary data will be collected from pilot plants while Ecoinvent 3.6 database will be used as secondary data source.

Eco-design practices will result in reducing the environmental footprint of SHJ-IBC technology compared to current technologies. Most appropriate materials and processes to close the loop on PV panel recycling will be suggested.

All this will contribute to rebuilding the “made in Europe” brand: using state-of-the-art technology which complies with the last environmental standards. Therefore, security of supply, affordability and a more sustainable future EU low-carbon energy system will be guaranteed.

#### **5.01.P-Mo347 Life Cycle Assessment of Four-terminal (4T) Perovskite-on-silicon Tandem PV Modules Using Different Recycling Routes**

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We have previously presented a comprehensive life cycle assessment (LCA) for large area (~ 2 m<sup>2</sup>) perovskite-on-silicon tandem PV modules with 30% power conversion efficiency (PCE), 30-year lifetime, and 0.5% annual degradation, manufactured in a gigawatt-scale production facility. In a subsequent step, the LCA results are updated to include a full inventory (LCI) that considers different end-of-life (EoL) management strategies and forward-looking energy matrices.

In this study, we focus on the end-of-life stage and perform a full Life Cycle Inventory (LCI) compilation through literature review and direct contact with industrial recyclers. To date and to our knowledge, this is the first study of its kind for four-terminal (4T) perovskite-on-silicon tandem recycling.

During the study, prototype four-terminal (4T) perovskite-on-silicon tandem solar modules are processed according to the proposed recycling routes and physicochemical analyses are performed throughout the process. The main results of this study are the separation efficiency of recycling technologies, the physicochemical properties of recycled materials, the environmental impacts of recycling processes, and the potential environmental credits of recycled materials. These credits are calculated using the cut-off approach.

We observed that for all impacts categories, advanced recycling processes show significant lower environmental impact, compared to the 1<sup>st</sup> generation recycling processes (reference case). Techno-economic assessment and experimental data will complement our study and further demonstrate the scalability and viability of advanced recycling technologies in terms of material sustainability, processing time and cost.

#### **5.01.P-Mo348 Life Cycle Assessment of Fibre Reinforced Composites from Retired Aircrafts**

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Many modern aircraft are built with a high percentage of plastic composites such as fiber-reinforced plastics (FRP) to achieve optimum light weighting whilst retaining robust performance. The use of FRP in aircraft frames and interiors helps to reduce fuel consumption and lower manufacturing cost compared to using metal. The excellent flame retardant properties of FRP make it a more favourable candidate compared to wood and bamboo. However, the use of FRPs make recycling at end-of-life very difficult and the circularity and carbon footprint should be measured to have a more informed understanding of the impact of materials choice on the environment. Aircraft are retired when they are no longer safe and economical to maintain; which usually equates to 20-30 years of service. Other external factors, such as the Covid-19 pandemic, have witnessed a decline in demand for some aircraft e.g., the Airbus A380 goes into early retirement after only 10 years in service. The increase in retired aircraft means boneyards are filling up with complex plastic-composites. This paper assesses the materials used in aircraft interiors to evaluate the potential of moving to a more circular economy through either recycling or reuse. In this study, various plastic parts from the cabin interior of an aircraft's fuselage (e.g., hinges, parts of the passenger safety unit, tubing, window pane, rim holder, panels and lid cover) were collected and analysed by size, colour, weight and polymer type through Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR). Among the predominant plastic materials identified are polyetherimide, polycarbonate, nylon, polyvinylchloride, silicon rubber, polymethyl-methacrylate, polysulfone and polyvinyl alcohol. Identification by ATR-FTIR is followed by life cycle assessment to understand the environmental hotspots and cost trade-offs of material choices and in this respect, the implications of waste management strategy to either recycling or reusing these parts.

#### **5.01.P-Mo349 Mineral Waste Recycling For Construction in Wallonia Using Life Cycle Assessment as an Eco-design Tool**

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Today's global needs for mineral resources are on a continuous increase, resulting in an intensification of their extraction and environmental pollution. In Wallonia (region of southern Belgium), those threats are shifting the industrial interests in the construction sector towards sustainability development plans based on reverse mineral industries.

The main challenge of this study is the selection of local mineral wastes from deconstruction to obtain materials with equal or superior properties and minimal environmental impacts.

Supported by research institutes, industries will conduct studies to spur the development of new sectors and value chains, under the umbrella of 'REMIND' program (Reverse Mineral Industry in Wallonia).

Thus, a case-by-case characterization of waste will be carried along with Life Cycle Assessment (LCA) as a decision-making tool to support the eco-design and the strategic alternative choices associated with targeted construction materials (insulating foams, concrete aggregates, self-compacting concrete materials, high-performance cement, and alternative binder materials for cement). An upscale approach will also be adapted to the potential market.

The strategy, including LCA, is expected to lead to new resource-saving and energy-saving technologies as well as alternative business opportunities that will turn into economic trends in Wallonia. LCA will be coupled to a rational material science approach in order to guide the optimization of materials with respect to the constraints of specific applications, involving the environmental impact as an objective to minimize.

The idea of processing mineral wastes will make a circular economy in the region possible to implement, which will not only improve the efficiency of industries but also have a strong ecological, social, and economic effect in the future.

#### **5.01.P-Mo350 Development of Environment Friendly Binders for Soil Treatment, Waterproofing and Roads Applications in the Walloon Region, Belgium (ECOLISER)**

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The building and construction sector plays a crucial role in our wellbeing. However, construction consumes more than 30% of the global resources and is responsible for the generation of more than 40% of the total solid waste volume. By consequence, there is a growing concern over making this sector more sustainable by the application of eco-design together with the improvement of resource efficiency and management.

The ECOLISER project aims to develop environment friendly binder formulations based on secondary materials and industrial byproducts (slag, blast furnace ash, fly ash, etc.).

This project's main objective is to minimize the impact of human activity on the environment in the Walloon region (Belgium), focusing on the industrial sector. The rehabilitation of brownfield sites into areas for new industries plays a significant role as well as limiting the landfill of industrial byproducts.

There are 3 types of alternative environment friendly binders targeted: for the stabilization of soils, for soil sealing and tightness, and the installation of reactive waterproofing barriers to fix heavy metals and micropollutants from percolating water and the production of cohesive materials for road infrastructure.

The first phase of this project is the global validation stage (experimental pilot site). Afterwards, a phase of constant evaluation of the performance of the technological solutions is applied in terms of sustainable development. This is achieved by using the life cycle assessment (LCA).

This LCA aims to study the potential environmental impacts produced by 1 km of road in Belgium. A road with hydrocarbon coating and a concrete road type are studied. These cases are compared to a road using the best proportion of the ECOLISER binders. In addition, to analyze the stabilization of soils upstream of road construction, LCAs are carried out for the conventional treatment of 3 types of soil: sandy, clayey and loamy.

The methods recommended by ILCD were chosen to evaluate the environmental impact of the road (European Commission JRC-IES, 2011). The modeling is performed in Simapro software, using Ecoinvent 3.9 databases.

Eco-design practices will result in reducing the environmental footprint of the ECOLISER binders compared to current products used in road construction.

Globally, and in a circular economy perspective, this project promotes one of the promising solutions to preserve natural resources as well as limiting the landfill of this byproducts.

#### **5.01.P-Mo351 Contextualized Phosphorus Recycling: Potential Diminution of Phosphorus Criticality at Territory Scale - Application to Agricultural LCA**

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Phosphate rock is classified as critical raw material for the European Union's economy. It provides phosphorus (P), which is an essential element for food production and is used as a mineral fertilizer. Due to a lack of a phosphorus substitute in fertilization, recycling is presently the only solution to reduce criticality, especially for countries such as France, that has no phosphate rock reserve and thus, must depend on foreign countries for its P needs for agricultural purposes. MAFOR or fertilizer mineral/organic matter from waste can contain available phosphorus. However, the recycling of P from these deposits for agriculture involves many actors (farmers, regulators, etc.) and depends on the studied spatial scale and its context (economic, regulatory, social, etc.) which can be either constraints or drivers for P recycling. Nevertheless, these issues are not taken into account in the recycling rate used in the raw material criticality assessment nor in the LCA methods. Therefore, it is necessary to contextualize P recycling for a better understanding of phosphorus recycling, and to allow a relevant phosphorus criticality assessment and an environmental impact assessment of phosphorus recycling at a local scale. In order to achieve this, a contextualized model is developed. The goal is to be able to quantify phosphorus which would potentially be used by farmers, originated from the total available quantities of deposits, by respecting the context of the studied territory. The originality of the model lies in the integration of context-factors that can affect the use of P contained in MAFOR by farmers. The model will be applied in two territories in France, which are different regarding the origin of MAFOR produced. As output of the model, four indicators are expected: the effectiveness and efficiency of phosphorus recycling, the dependence on phosphate mineral fertilizers imported from outside France and the contextualized balance between supply and demand in P recycling-derived fertilizers. The model gives a methodology advancement, which would improve or be a complement of the LCA tool to assess territorialized recycling scenarios. The output indicators would improve the understanding of P circularity and, help determine a suitable P recycling pathway to reduce P criticality at a territorial scale and relevant from an environmental point of view.

#### **5.01.P-Mo352 Effect of Hazardous Waste Landfill Leachate on Bentonite Barrier Stability**

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Although the number of landfills for solid waste disposal is going to decrease, some waste will still be landfilled, especially toxic waste including incinerator fly ash. The structural elements of landfills often include sealing geosynthetic clay (bentonite) liners to separate solid waste from the subsurface environment. During the period of productive activity, the waste material creates suitable conditions for different microbial communities and generates heat. This often creates highly aggressive substances, accompanied by increased temperature affecting the functionality of sealing geosynthetic clay liners. Our study aims to determine bacterial populations that increase in the bentonite saturated with hazardous waste landfill leachate and whether this impacts the bentonite's isolation properties.

Freshly collected landfill leachate from the hazardous waste landfill was transported to the laboratory and mixed with bentonite. Mixtures were airtight and incubated at 50 °C for three months in a self-developed anaerobic atmosphere. As the controls, vessels containing bentonite mixed with tap water and sterilized (Gamma irradiated) landfill leachate were added to the same heated box. At the end of the incubation, samples were collected for DNA extraction and tests of bentonite's chemical, geo-mechanical and hydro-dynamical properties. Extracted DNA samples were used for qPCR and NGS analyses to compare the abundance and composition of bacterial communities in bentonite mixtures.

Most bentonite samples had higher bacterial abundance after incubation compared to the beginning of the experiment. Bentonite mixed with the landfill leachate contained high amounts of acetogenic, iron-reducing, and sulphate-reducing bacteria. The physical properties of bentonite saturated with the leachate deteriorated dramatically compared to the control with tap water. A decrease in the bentonite sorption and swelling capacity could be caused by the high salinity of the landfill leachate. The extreme chemical properties of hazardous landfill leachate with the high activity of microorganisms can influence the bentonite sorption capacity and its isolation properties. This can lead to the leakage of toxic substances into subsoil and groundwater. This phenomenon could probably be avoided or reduced by proper pre-hydration of bentonite with fresh water during landfill construction.

#### 5.01.P-Mo354 Effectiveness of Layered Wearing for Mitigating GHG Emissions

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The Ministry of the Environment of Japan has adopted "Warm Biz" as a measure to combat global warming. By wearing layered clothing in winter, room temperature can be set lower. However, few studies have examined the relationship between how clothing is worn and its effectiveness in reducing greenhouse gas (GHG) emissions. Therefore, this study aims to quantitatively calculate the GHG emission reductions from layering clothing by using life cycle assessment (LCA). PMV, an index of thermal comfort, was used to determine the set temperature of heating equipment in the house. The clo values used in the PMV equation were measured using thermal manikins and values cited in the literature. For the rest, the metabolic rate was 58.2 W/m<sup>2</sup>, air velocity was 0.1 m/s, radiant temperature was 20.0°C, external work was 0.0 W/m<sup>2</sup>, and relative humidity was 50.0% to obtain room temperature at which PMV = 0. Internet Questionnaire Survey was conducted to clarify the use of heating equipment and clothing in the home. A total of 2,000 people living in Hokkaido, Miyagi, Tokyo, Osaka, and Fukuoka prefectures were targeted. The survey included the type, temperature setting, duration, and time of use of heating equipment, the number and useful life of winter clothing and heat-retaining underwear, and the time spent in the house before and after COVID-19. The system boundary is assumed to be from the manufacture of the garment to its wearing (air conditioning use), washing, and disposal. GHG emissions over the entire life cycle were calculated for one year. Based on the results of Internet Questionnaire Survey, clothing in Tokyo residences is assumed to be worn: a cloak, sweatshirt top, sweatshirt bottom, underwear, and socks. The calculation results will be discussed on the day of the presentation. It was found that GHG emissions from heating equipment use account for the majority of GHG emissions over the entire life cycle.

#### 5.01.P-Mo355 An Integrated Strategy to Address the Biodegradability of Cosmetic Formulations as Part of a Corporate Sustainability Initiative

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Biodegradability of organic chemicals usually disposed-off down-the-drain in their normal life cycle is a key parameter to be considered by industries pursuing sustainability in their new product developments. It is a property that, once improved in any formulated product portfolio, will enable a significant decrease in the presence of its ingredients in the natural environments, with a potential reduction in the pollution related post-consumption environmental impact as a consequence. Considering the importance of biodegradability as an indicator of product environmental performance and the lack of internationally recognized standards for complex mixtures, the present article aimed to propose an intelligent strategy to address this parameter in the cosmetic sector, with possibility to support both product claims and corporate sustainability initiatives. A case study is presented and discussed in detail, as a proof of applicability of the whole framework to support new developments towards improved environmental performance in terms of chemical persistence. Important insights in terms of ingredients assessment criteria are also presented.

#### 5.01.V Circularity, Recycling and Multi-Functional Systems

##### 5.01.V-01 Human Toxicity Assessment of Waste Electric and Electronic Equipments (WEEE) Recycling Process

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The generation of Waste Electric and Electronic Equipment (WEEE) is growing quickly, producing vast amounts that are not sustainably disposed. WEEE consists of a considerable portion of plastic containing harmful chemicals, like legacy brominated flame retardants (BFRs), which are not easily recycled. With the aim to deal with WEEEs, the European research project Plast2BCleaned developed an innovative dissolution-based recycling process. The process requires the use of chemicals and demands that all workers who have contact with such chemicals follow applicable health & safety measures. However, there is currently a lack of data for most chemicals regarding human toxicity during their use phase, which creates an information gap in Life Cycle Assessment studies that consider the human toxicity of these chemicals. Thus, the goal of this study was to assess human toxicity impacts on plant workers during the Plast2BCleaned recycling process, as a complementary aspect to assessing life cycle impacts of WEEE recycling. Exposure and potential toxicity effects were identified for relevant steps of the process, such as during the filling of a solvent (to be mixed with plastics during the dissolution process) and during storage of the final products (where the chemicals are emitted in the air in a closed environment for a limited time). The USEtox scientific consensus model was used to determine the impacts of this exposure for humans working in the process, by adapting underlying aspects for direct emissions and for chemicals released from materials for the three selected solvents. The concentration in air,

characterisation factors and impact scores for each chemical in different steps of the process were determined. Endpoint impact results (expressed in Disability Adjusted Life Years - DALYs) for different solvents and BFRs were obtained. The results show that all three chemicals have their concentration levels below the regulated Threshold Limit Values (TLVs). The estimated emissions of solvents to the air during the filling process and in a spillover scenario, exceeds the TLVs by a factor of 5 to 10. However, in the study conservative time for the filling was assumed and in the real scenario safe short term exposure limit is not exceeded. The endpoint impact results for human toxicity in terms of DALYs are very low for the given worker's exposure time to the chemicals. Our study is a starting point for combining direct worker effects with life cycle impacts.

#### **5.01.V-02 Combination of Life Cycle Assessment and Process Simulation to Improve Recycling Processes and Scale-up**

**Yvonne Kaye Perocillo<sup>1</sup>** and **Angelique Leonard<sup>2</sup>**, (1)University of Liege - Chemical Engineering, Belgium, (2)University of Liege, Belgium

In this time of increasing demand for raw materials, and environmental awareness, designing an efficient metal recycling process is increasingly becoming critical in achieving a more circular-oriented economy. Despite numerous literatures of successful processes developed for specific waste materials, there are only a few that have been applied in the industry. The challenge lies not just in the technical viability of the process upon scale-up, but also on the possible burden shifting of environmental impacts due to said process. Using life cycle assessment (LCA) to assess these impacts is necessary. But since LCA was initially developed to assess a system's primary function, dealing with multifunctional systems like a recycling process is only based on the practitioner's discretion thus needing improvement. Scaling up the process is also technically challenging because of the great variation in the process' efficiency and performance in the laboratory and when scaled up. Though process simulators give perspective on how the process performs in terms of products and material or energy flows, effects of equipment size and design are not necessarily considered on most simulators. This research aims to address these challenges in both LCA and process simulation through case studies of several types of recycling metal loops for both experimental and industrial metal recycling processes especially those dealing with end-of-life for electromobilities.

#### **5.02 Guidance for LCA of Recycling Abiotic and Biobased Materials and for the Transition to a Resilient Low Carbon, Resource-Efficient and Circular Economy**

##### **5.02.T-01 A Guideline for Life Cycle Assessment of Carbon Capture and Utilization as Negative Emissions Technologies**

**Sibylle Duval--Dachary<sup>1,2</sup>**, **Amandine Pastor<sup>2</sup>**, **Sandra Beauchet<sup>1</sup>**, **Daphné Lorne<sup>1</sup>**, **Thibault Salou<sup>2</sup>** and **Arnaud Helias<sup>2</sup>**, (1)IFP Energies Nouvelles, France, (2)ITAP, Univ Montpellier, INRAE, Institut Agro, France

Moving towards a circular carbon economy would reduce the extraction of additional fossil raw materials. Carbon capture and utilization (CCU) technologies could thus reduce greenhouse gases emissions by replacing fossil-based products by CO<sub>2</sub>-based products (avoided emissions). In this study, we focus on CCUNET: CCU systems combining these avoided emissions with negative emissions technologies (NET). Quantifying the whole environmental impacts (both avoided and negative emissions) is complex. Guidelines to harmonize LCAs of CCU systems and improve results comparability between several studies exist. However, the coupling with NET generates issues on, for instance, system boundaries or biogenic carbon accounting. To address these concerns, existing recommendations to perform LCA on CCU or NET systems are identified and discussed. This allows proposing tailored recommendations for LCA of CCUNET systems. The guideline addresses, among others, the choice of i) the functional unit, ii) the system boundaries, iii) the method to deal with multifunctionality, and iv) the integration of biogenic carbon flows, including soil carbon sequestration. For instance, the functional unit should consider carbon sequestration to allow comparison with other NET. A case study is modelled to exemplify this guideline. To be able to evaluate negative emissions, the system boundaries must be cradle-to-grave, i.e. from CO<sub>2</sub> removals from the atmosphere to the end-of-life of the CO<sub>2</sub>-based product. Thus, the case study includes i) biomass production (CO<sub>2</sub> removal from the atmosphere by photosynthesis during biomass growth), ii) conversion of the biomass into energy and capture of the produced CO<sub>2</sub>, iii) transformation of the captured CO<sub>2</sub> into a value-added product, iv) use of the CO<sub>2</sub>-based product, v) end of life of the CO<sub>2</sub>-based product. For instance, it could be CO<sub>2</sub> captured in a fuel ethanol plant supplied by corn, miscanthus or even wood residues. This CO<sub>2</sub> will then be transformed into a reusable plastic bag. At its end-of-life, the plastic bag can be recycled, landfilled, or incinerated with carbon capture and storage. This LCA study innovates by proposing to model a complete CCUNET system and provide some first insights on the potential of CCUNET system to reach negative emissions. This framework is interesting in view of the growing interest in both CCU and NET systems, that will hopefully result in more and more LCA on CCUNET systems.

##### **5.02.T-02 Future Greenhouse Gas Emissions from Metal Production and their Relationship with Climate Goals**

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Metals play an essential role in human life, while metal use is associated with not only metal depletion but also environmental concerns. To discuss strategies towards sustainable metal use with lower environmental impacts in line with climate goals, quantifying future environmental impacts from metal production and exploring effective measures for alleviating the environmental impacts are essential. Therefore, we estimated the global greenhouse gas (GHG) emissions from the future production of six typical metals (aluminum, copper, iron, lead, nickel, and zinc) under the five shared socioeconomic pathways (SSPs) for 2010-2100 and compared the results with a GHG emission reduction target (2°C target). In addition, we explored the influential parameters of metal cycles to reduce the environmental impacts by scenario analysis.

We show that trends for GHG emissions from metal production are significantly different among SSPs, while the 2°C target will not be achieved for the metal sector under any SSP, mainly due to the increase in GHG emissions in the early 21st century in

middle-income countries. This suggests that substantial efforts to reduce GHG emissions are required in addition to the transition to the sustainable socioeconomic pathway. From a short-term perspective, lowering the per capita in-use metal stock level and GHG emission intensity of metal production is identified to be effective. From a long-term perspective, improving the recycling rate will also be an effective way. However, our analysis shows improving a single parameter is expected to be insufficient for achieving the 2°C target. Given that GHG emissions from metal production will increase mainly in the early century and improving parameters cannot be achieved promptly, implementing multiple measures immediately with international cooperation, as well as following the sustainable socioeconomic pathway, is essential for sustainable metal use in line with the climate goals.

#### **5.02.T-03 Life Cycle Assessment of Biogenic Carbon Capture and Storage Processes: Review of Life Cycle Inventories and Recommendations**

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Bioenergy with carbon capture and storage (BECCS) are part of the solution to reduce greenhouse gas emissions into the air. The idea is to capture biogenic carbon before it is emitted into the atmosphere and stored permanently. If biogenic carbon is stored indefinitely, it generates a flow of carbon from the atmosphere to a permanent sequestration. Thus, BECCS systems are considered as negative emission technologies (NET). However, in seeking to reduce the climate change impacts of BECCS systems, the technologies that comprise the BECCS system may impact other environmental phenomena. This may result in pollution displacement. Different technologies are available at each stage of the life cycle of a BECCS system. It is important to provide the decision maker with a clear understanding of the environmental impacts associated with the choice of these technologies. The objective of this study is to review the quality of the life cycle inventory data available in the literature and to provide recommendations to conduct a quality BECCS Life Cycle Inventory. An analysis of 35 LCA studies was conducted. These studies had to propose a BECCS life cycle inventory. The BECCS technologies considered start in the field with the cultivation of the biomass (depending on the type of biomass) and its harvesting. Several technologies can then be used to ensure the pre-treatment of this biomass, its combustion and gasification, and finally the capture of the biogenic CO<sub>2</sub> emitted. Afterwards, the CO<sub>2</sub> can be compressed for transport and then stored. Only 13 of the 35 articles reviewed provide life cycle inventory tables. Inventory data is often marked by a lack of reproducibility, of data. Nevertheless, this study provides LCI data and recommendations for conducting LCIs to facilitate harmonization between future LCAs on the BECCS. It is important that the inventory data be transparent with a clear display of boundaries and system assumptions. It would also be interesting for BECCS LCI to define the temporality of the data and their geographical representation. Each data quality criteria must be discussed as recommended by ISO 14 040/44.

#### **5.02.T-04 Operationalizing the Geopolitical Supply Risk Potential as a Supply Risk Indicator for Use in Life Cycle Assessment**

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The geopolitical supply risk (GeoPolRisk) method evaluates raw materials criticality in Life Cycle Assessment, complementing other resource use and environmental impact indicators. However, calculating characterization factors (CFs) for the Geopolitical Supply Risk Potential as a supply risk indicator at midpoint level for use in life cycle assessment, (GeoPolRisk midpoint) is challenging. Therefore, we developed a calculation library to operationalize the GeoPolRisk method to fulfil two functions: 1) to facilitate the calculation of the CFs for the GeoPolRisk midpoint indicator and 2) to showcase its application as a comparative risk assessment. The geopolrisk-py is a python-based library containing functions in multiple modules providing the users a wide array of options. Users can provide unique trade data for a company/organization level assessment with the library for more specific values. The library also hosts functions that allow users to extract and visualize results. The structure of the library and its functioning has been demonstrated in multiple case studies. The application of the tool is presented through integrated LCA study with GeoPolRisk of an electric car and a comparative risk assessment case of cobalt imports to three companies in the United States of America, Sweden, and South Korea. Finally, a web tool has been developed with the library as its framework to conduct an assessment using a graphical user interface. The CFs for the GeoPolRisk midpoint indicators are available to download at request from the web tool.

#### **5.02.T-05 A Host-to-Byproducts Matrix to Help Assessing Abiotic Resources Accessibility**

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There is a growing concern about our current unsustainable management of abiotic resources over their entire life cycle. Operational life cycle impact assessment (LCIA) methods consider that making a resource inaccessible has a potential impact, regardless of its functionality and the future demand of services provided by the resource. However, the extent of damages of a resource made inaccessible i.e. dissipated, with (or without) substitutes and with low/high demand for the services it provides may be very different. A LCIA framework has been developed by Greffe et al. (2022) to link resource dissipation to the deficit of services. To operationalize the fate model of the LCIA framework, a dynamic material flow analysis under constraints has been developed. It traces the fate of resources and materials from the extraction from the accessible to stock to the in-use stock (with recycling flows). It proposes to assess the mismatch between demand and supply of resources in the in-use stock. The flows of resources from one stock to another are constrained by a maximum yearly production. Most metals used in a wide range of products are extracted as byproducts of hosts, meaning their accessibility depends on the demand of host elements, such as zinc, iron, aluminum or nickel. Although plenty of estimates of metals content in different sources such as mineral ores, slags or

tailings are available, there is currently no harmonized dataset that enable to answer to the question: what is the average quantity of byproduct b potentially recoverable with regards to the extraction of host h? Our current work aims at answering to such question.

We present a consistent methodology to calculate host-to-byproducts ratio for all abiotic resources used in the economy. We harmonize the data from the literature and we will present our preliminary results for dozens of metals, with a confidence interval (5th and 95th percentile). This matrix can then be used in material flow analysis of multiple resources to model their fate in the economy. The host-to-byproducts matrix will enable to determine the flows of byproducts that will be accessible to fulfill services to humans. It will then be possible to characterize the impact of dissipation of resources, applying the LCIA framework of Greffe et al. (2022).

## **5.02.P Guidance for LCA of Recycling Abiotic and Biobased Materials and for the Transition to a Resilient Low Carbon, Resource-Efficient and Circular Economy**

### **5.02.P-Mo356 The Importance of Land in a Low Carbon and Bio-based Economy: Quantifying Supply Risk in a Life Cycle Perspective**

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The transition toward a low-carbon economy relies on the use of renewable energy and bio-based material. These systems require large amount of land, such as photovoltaic system or industrial crops, adding pressure on the resource. Although acknowledged as a key resource, land has not been included in criticality assessments, in particular in the recent criticality methods developed for inclusion in life cycle assessment (LCA), which use the supply risk (SR) index, reflecting the possibility of supply disruption for a given material based on its geological, economic or geopolitical availability, to develop new characterisation factors. This study therefore aims at developing country level land supply risk (SR) indexes in line with two existing criticality methods for other resources. These developments should allow for the quantification of risks related to potential inaccessibility of land resources and for disparities between countries to be revealed.

To extend the SR framework to land resources, every SR component for mineral resources is adapted to land in both methods, when appropriate. This approach ensures comparability across resources. The developed land SR are computed at the country level to consider local drivers influencing land accessibility. The main adaptations include the definition of a land stress index, representing the physical availability of land, and an internal land concentration index, relating to the concentration of landowners within a country.

Finally, land supply risk indexes are computed for 76 countries, including 24 European countries for which the results of the two criticality methods are compared. Comparison points to divergences in the country ranking for land accessibility, thus underlining the importance of methodological choices in the construction of the indexes.

The results of this study showed that the criticality framework could successfully be applied to the land resource, highlighting the various factors that can prevent access to land in different countries. Yet, the model could be further improved by focusing on finer distinctions between types of land use and taking into account land quality. The current integration of the criticality concept in LCA could benefit from these land supply risk indexes, which could be used to derive characterisation factors for future LCA criticality assessments.

### **5.02.P-Mo357 Greenhouse Gas Removals Via Biochar Production From Food Waste Digestate in the UK**

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Biochar can potentially contribute as a Greenhouse Gas Removal (GGR) technology to achieve UK's net zero emission target by 2050, but risks being constrained by the availability of conventional feedstocks such as wood. Biomass wastes which are less attractive in energy applications, particularly anaerobic digestate, can extend the scale of biochar deployment. Application of stable biochar in the soil is capable of achieving a long-term carbon storage with potential co-benefits for improving the soil quality. Hydrothermal carbonisation, with subsequent high temperature torrefaction, is capable of directly producing stable biochar from wet wastes such as digestates. In this work we evaluate the potential scale of biochar production from anaerobic digestates in the UK for 2030 and assess the techno-economic and life cycle environmental viability of this approach.

Anaerobic digestion (AD) plants treat numerous feedstocks including the current input of around 30% is from the food waste; in 2019, over 4 million tonnes of food waste were treated in the AD plants. Future growth in the sector will be driven by food waste management, where AD is viewed as an environmentally favourable treatment method for unavoidable food waste. Our analyses indicate the biochar production from food waste digestate achieving a substantial net greenhouse gas (GHG) removals of approximately 1.6 t-CO<sub>2</sub>eq.t-biochar<sup>-1</sup>. Transport distance and soil effects are uncertain but are estimated to have a small impact on GHG emissions, highlighting that the majority of emissions reductions are from the physical storage of carbon in biochar (1.9 t-CO<sub>2</sub>eq.t-biochar<sup>-1</sup>). The use of 50% of UK's projected available food waste digestate by 2030 can sequester around 85 kt-CO<sub>2</sub>eq p.a., requiring 28 individual 20 kt p.a. biochar production facilities. Commercial biochar production from food waste digestate is able to provide cost-effective GHG removals of less than £100 t-CO<sub>2</sub><sup>-1</sup> avoided. Other wet wastes such as green waste will command a lower gate fee resulting in higher costs of avoiding CO<sub>2</sub>. Sensitivity analysis demonstrates the heavy influence of the gate fee and its importance for biochar process establishment. This work considers a highly promising opportunity in solving a



waste disposal burden and simultaneously removing atmospheric GHGs. Multiple sources of biochar will be needed to make significant contributions to the UK's GGR target.

**5.02.P-Mo358 Life-Cycle Approach in the Valorisation of Maize Bran for the Biotechnological Production of Ferulic Acid**  
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Agro-industrial residues provide a great source of sugars, pigments, proteins, antioxidants, etc. Among the group of polyphenols, ferulic acid offers potent antioxidant activity, and excellent antimicrobial and antifungal activity. In this work, two bio-based strategies to produce ferulic acid (FA) from maize bran were evaluated from an environmental approach. The aim is to determine those aspects that can hinder the viability of these valorisation routes, and thus, to establish improvement strategies during the early stage of the process. The Life Cycle Assessment (LCA) methodology is used to determine the environmental performance of bio-based FA production, through a life cycle perspective and following ISO 14040 and 14044 guidelines. The functional unit corresponds to 1 kg of PA, based on a "cradle to gate" approach. FA production was modelled in Superpro designer® v11 software, considering a small-scale biorefinery located in Egypt. The plant provides a maize bran capacity of 16,350 t·y<sup>-1</sup>. The production stages were pre-treatment (to obtain de-starched maize bran), heat treatment (steam explosion or autoclaving), enzymatic hydrolysis and downstream processes. The first scenario (SET) corresponds to a steam explosion followed by enzymatic hydrolysis followed by enzymatic hydrolysis by adding 3% w/w protein/bran of enzyme (Novozyme 342) for 24 h at 40°C. In contrast, the second scenario (AUT) considers an autoclave treatment followed by enzymatic hydrolysis with 1% enzyme (feruloyl esterase, FAEA) for 16 h at 40°C. The pre-treatment and subsequent processes were the same for both routes. To obtain the environmental profiles, the characterization factors of the ReCiPe Midpoint v1.07 (H) method and SimaPro 9.1 software were used. In addition, background process inventory data were obtained from the Ecoinvent® v3.8 database. Global Warming (GWP), Freshwater Eutrophication (FE) and Freshwater Ecotoxicity (FEC) were some of the impact categories evaluated. Results indicate that the AUT strategy obtains the best environmental performance in all categories. Highlighted results for the AUT scenario (related to the SET strategy) were observed in FEC and FE categories with a reduction of about 57% and 50%, respectively. Moreover, the GW profile of both strategies was 660 and 432 kg CO<sub>2</sub>eq per kg of FA for SET and AUT, respectively. The results can be explained due to the FA content present in the maize bran (3.1% w/w) and the intense heat demand of the process.

**5.02.P-Mo359 Reviewing the Life Cycle Assessment of Engineered Wood Products in the Built Environment: Gaps, Challenges and Opportunities for Improvement**

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The built environment accounts for approximately 37% of the global carbon emissions. As buildings become more energy efficient, embodied carbon is becoming the target of multiple efforts to transition the built environment towards more sustainable practices. The use of engineered wood products (EWPs) presents opportunities in the construction sector by meeting strict quality and structural properties while presenting a low embodied carbon profile and the capacity to store carbon for a long period of time. Environmental implications of product or services systems is usually assessed with Life Cycle Assessment (LCA) studies. LCA is an internationally standardized method that allows to translate the resource consumption and emissions associated with a product system, into potential environmental impacts. In order to compare the environmental implications of building with conventional materials vs. engineered wood products, LCA studies rely on inventory databases, EPDs, and available impact assessment methods to compare between the alternatives assessed. However, recent studies have pointed out the limitations observed when conducting LCA studies in compliance with European Norms in regards to the environmental benefits of long-term carbon storage provided by wood products. Besides these limitations, several differences, both in inventory and impact assessment, regarding bio- and conventional materials, might be hampering an accurate representation of the impacts and benefits associated with the use of EWPs. To determine if methodological and/or operational characteristics should be improved for a robust assessment of EWP in the built environment, this study targets the assessment of EWPs in LCA by providing an overview of identified gaps and recommendations to further develop and improve their assessment. The findings are expected to contribute to the body of knowledge covering circularity and biomaterials in the built environment, as well as efforts to improve the impact characterisation and impact assessment methods used in LCA.

**5.02.P-Mo360 ALIGNED: A framework for the LCA of Bio-based Products**

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The assessment of bio based products presents several challenges: from the definition of system boundaries and choice of system models, modeling competition for biomass and for land, to performing dynamic and time specific accounting, making scenarios and considering uncertainty.

We present the background, methodology, and expected results of the Horizon-Europe-funded project "Aligning Life Cycle Assessment methods and bio-based sectors for improved environmental performance" (ALIGNED, grant number 101059430). Targeting five sectors namely woodworking, pulp and paper, biochemicals, construction and textiles, ALIGNED fulfils three

research needs: 1) to improve, harmonize, and align LCA methodology for the assessment of bio-based products covering environmental and socio-economic aspects, 2) to demonstrate the harmonized methodology to improve the environmental performance of specific technology development cases in industries within the bio-based sectors 3) to inform and involve stakeholders, enabling an efficient methodological uptake.

ALIGNED modelling framework does not intend to provide a new standard or guideline but instead to make available an ecosystem of science-based and open approaches and tools to ease the assessment of bio-based products. Key elements in such framework include: 1) A science and evidence-based approach: scientifically sound modelling as close as possible to reality, avoiding normative rules, and favoring models that can be validated and revised when new data become available as well as an ecosystem of interacting models and tools. 2) A life cycle perspective: the assessment takes the full life cycle of bio-based products into account. 3) Relevance for decision-makers and usefulness for decision support: we focus on modelling what are the consequences of specific decisions. We explicitly consider uncertainty in the decision support. 4) Balancing model complexity and model applicability: we select and use in this framework models that are scientifically robust but also usable by practitioners to the largest extent, keeping in mind the trade-off between model complexity and applicability. 5) Adherence to open-science practices: models, tools, and their documentation are open, while data should be as open as possible. 6) Ensuring relevance for bio-based products: the modelling framework considers the specific challenges and issues that exist in the assessment of bio-based products.

## **5.02.P-Mo361 Application and Analysis of Different Methodologies for Modelling Temporal Carbon Sequestration of Biobased Materials**

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Biobased materials can reduce the Global Warming Potential (GWP) of products when substituting other materials and have the chance to sequester carbon for their useful life, thus reducing the total amount of atmospheric Green House Gases (GHG) temporary. State of the art Life Cycle Assessment (LCA) does not take into account the effects of temporal biogenic carbon sequestration and the time of release of GHGs, thus not representing differences due to the expected useful life and cascade use of the material. Multiple approaches exist to model these differences connected to temporal carbon sequestration such as the static approach proposed in the ILCD handbook or dynamic approaches such as dynamic LCA, GWP bio or a Climate Tipping Point Metric. Even though there is a consensus in scientific literature that it is important to model such temporal effects, these modelling approaches are only seldom applied and there is no consensus on which modelling approach is the most feasible to be used in LCA studies. Within this work, different approaches for the inclusion of temporal carbon sequestration will be applied for two different use cases for biobased materials with scenarios for multiple life cycles: a) the application of a wood-based door impact beam in a car with different scenarios for the useful life and b) the application of a wood-based foam made from primary and secondary material for a surf board. The methodologies will then be analyzed regarding ease of use, availability and reliability of data and implications for the decision making processes, thus showcasing problems and chances with the application of the different methodologies and advancing the discussion of temporal carbon sequestration within the field of LCA.

## **5.02.V Guidance for LCA of Recycling Abiotic and Biobased Materials and for the Transition to a Resilient Low Carbon, Resource-Efficient and Circular Economy**

### **5.02.V-01 Is Circular Economy Truly Sustainable? Robust Comparative LCA of Circular Pavement Designs Using a Probabilistic Approach**

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As global climate change becomes evident, reducing CO<sub>2</sub> emissions is regarded as one of the most pressing issues for our industry and society. The asphalt pavement industry is also identified as one of the important CO<sub>2</sub> emission sources. Life cycle assessment (LCA) has been widely used to quantify the environmental benefits of designs or practices that can improve the sustainability of asphalt pavements, such as reusing end-of-life pavement materials in new asphalt mixtures. However, due to the paucity of representative and good quality data, LCA results of pavements are associated with considerable uncertainty, thus unable to ascertain the benefits of sustainable designs or practices.

Against this backdrop, this study develops parametric models to characterize energy uses and CO<sub>2</sub> emissions occurring in key life cycle stages (including bitumen production, asphalt mixing, and pavement construction). A probabilistic approach is employed to quantify the uncertainty of input parameters and LCA outputs under three scenarios: (1) Baseline, (2) 0% RAP, and (3) +4cm durability.

The results indicate that reusing end-of-life pavement materials does not necessarily reduce the CO<sub>2</sub> emissions of a pavement over its life cycle. The CO<sub>2</sub> benefits of reclaimed asphalt pavement (RAP) depend on the durability of asphalt pavements. More interestingly, the certainty of conclusions varies across comparisons. When comparing the Baseline scenario (in which RAP accounts for 27% of asphalt mixture compatible with Belgium's construction requirements) with the +4cm durability scenario (in which more RAP is used to compensate for durability loss), we are highly confident that the former leads to less CO<sub>2</sub> emissions. However, when comparing the Baseline scenario with the 0% RAP scenario (in which no RAP is used), we are less confident that the former leads to less CO<sub>2</sub> emissions. In addition, the results reveal that transport contributes the most to the uncertainty of LCA outputs, suggesting that more high-quality data on transport is needed to improve the accuracy of LCA results and the confidence in the conclusions drawn from comparative LCA.

## 5.03.A LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Comprehensive Analysis

### 5.03.A.T-01 Assessment of Sustainability Labels in the Context of a Life Cycle Assessment

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During the last few years, scientific publications on environmental labeling have substantially increased in number, which is an indication of greater international interest in mitigating environmental degradation, as well as with the increase in political and technical-scientific conferences on the subject. Labels should provide consumers with simple and catchy information on sustainability, nutritional and health aspects.

The objective of this study is to assess critically some selected labeling systems in terms of completeness, double counting and reliability. Special emphasis is placed on how method developers incorporate life-cycle thinking into their design. This study is based on a literature review of studies focusing on sustainability scores, including—beyond peer-reviewed articles—specific reports on sustainability scores that are publicly available.

An evaluation of various sustainability labels revealed several deficiencies regarding its scientific basis. The analysis showed that most labeling systems profit from a clear and catchy visual declaration. However, a closer examination of sustainability labels has shown that their scientific basis and completeness are questionable in some cases. Awarding additional points to food products that have already been awarded by another label from a third-party organisation was also judged critically, as this procedure increases the probability of double-counting.

We conclude that the enhancement of labeling beyond the LCA is critical, as this may lead to double-counting, green-washing and a lack of transparency. The elaboration of new labels should always follow strict scientific rules to avoid subjective weighting and redundancy. Modifications and expansions of labels based on the preferences of stakeholders and organisations should be avoided. Further, it is recommended to develop the LCA if necessary to enable the calculation of labels using a single method, thereby avoiding the implementation of workarounds.

In summary, it is crucial to inform consumers in a transparent and easily understandable way about gaps and weaknesses in the labelling scheme. Currently, however, European sustainability labels for products (such as the Eco-Score or Planet-Score) tend to overweigh certain aspects, without a sound scientific basis. It is strongly recommended to provide additional information, such as production standards and origin as separate information.

### 5.03.A.T-02 Enabling a Holistic Assessment for Foodware and Food Packaging to Protect Environmental and Human Health

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In an ideal world, foodware and food packaging enable circular and fair business models, do not have adverse impacts that destabilize the planet's ecosystems, and internalize the costs they have on humans and the natural environment we coexist with and depend on. However, in reality, the foodware and food packaging used to enable today's highly globalized, increasingly convenience-focused food system is being ever more identified as a source of damage to both human and environmental health. Currently, what stakeholders assess and how they assess it is neither consistent nor comprehensive. Different sustainability criteria are being applied across assessments and often with a narrow focus on single-impact areas, such as greenhouse gas emissions.

To enable a more holistic assessment and procurement of truly sustainable foodware and food packaging products, decision-makers need science-based guidance and tools. To support this, we have developed the Understanding Packaging (UP) Scorecard as an open access, transparent, and LCA-based calculation methodology and tool to help decision-makers compare over 100 generic foodware and food packaging products across six metrics: climate change, water use, plastic pollution, sustainable sourcing, recoverability, and chemicals of concern. Raw emissions as well as normalized scores are calculated for each metric, and all metrics are further weighted equally to define a single, normalized summary score.

The results of the UP Scorecard's assessment across a wide range of generic products reveal some clear trends that can help procurement professionals better consider a wider array of environmental and human health impacts when selecting products. This includes achieving the lowest impacts through implementing reuse systems, while single-use plastic products are often associated with the overall highest impacts.

This methodology aims to expand the impact areas considered within the current life cycle and sustainability assessments for foodware and food packaging. Given the complexity of materials used as well as the global diversity of (un)available infrastructure to manage end-of-life treatments for these products, the novel qualitative metrics included here can become more robust and accurate pending better availability of the underlying data. Additional impact areas could also be implemented within this methodology to even more holistically assess products, such as contributions to biodiversity loss.

### 5.03.A.T-03 Consequential Life Cycle Assessment of a Diet Change Following Planetary Boundaries Recommendations

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Environmentally friendlier diets are needed and at the same time health issues linked to a non adequate nutrition are rising. The aim of the planetary health diet developed by the EAT Lancet commission is to sustainably feed 10 billion people in 2050 while not exceeding planetary limits. According to these guidelines, a switch between legumes and red meat consumption is desired for European citizens. To evaluate environmental and economic consequences of this diet change, a partial economic model was combined with Life Cycle Assessment. A scenario of 80% decrease in red meat consumption in Europe in 2030 was simulated in order to assess crop and animal production changes associated with the diet change. The major output from the partial economic

model was the significant gap between consumption and production of red meat and legumes. Indeed, European production was only slightly changed and overstock of red meat or missing stock of legumes were respectively exported or imported. Additionally, the partial economic model allows to capture indirect changes in agricultural production. These direct and indirect changes in production and trades were used as inputs of Life Cycle Assessment. Using Environmental Footprint 3.0 methodology, the environmental assessment showed benefits for all impact categories. The desalignment between production and consumption in Europe would potentially have several negative consequences such as Europe dependancy and vulnerability towards big economic players or social consequences for farmers where exports take place. Changes in consumers behavior is not enough and strong policies targeting production such as incentives for decreasing meat production or subsidies to encourage transition to legumes production are needed. Overall, our results contribute to a better understanding of economic and environmental consequences of diet changes and to implied recommendations for policies.

#### **5.03.A.T-04 Revisiting the Territorial Life Cycle Assessment Framework to Account for Ecosystem Services**

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At the core of the territorial life cycle assessment T-LCA framework lies the idea that indicators of provided services be quantified to account for the multifunctionality of territories. This poses an innovative basis for the inclusion of the ecosystem services (ES) concept in life cycle assessment (LCA). By revisiting the T-LCA framework, a new methodological framework for environmental land planning is proposed. The academic literature was explored and revealed three general approaches for the inclusion of ES in LCA. These approaches were consistently analyzed and combined in a comprehensive novel framework. The analysis shows that incorporating ES at the different phases of a LCA can be done coherently as long as spatial and temporal concerns are taken into consideration. In the goal and scope phase, ES are considered as services offered by the techno-ecological system that is the territory. Here the focus is on the foreground system and all types of ES can be considered. For regulation and maintenance type of ES, the incorporation into the framework can occur at the life cycle inventory step, as they mediate the emission of certain substances in the environment. At last, all types of ES can also be considered within an area of protection at the life cycle impact assessment step, by developing models that quantify the impact of emissions or resource use on the potential future delivery of those ES for both the foreground and background systems. Impacts on ES generated the territory can be considered along with the ES of the functional unit without generating any double counting issue since the environmental impact would be measured on the potential future delivery of ES while ES considered in the G&S phase represent the actual delivery at the time the assessment is carried. In conclusion, combining the inclusion of ES at different steps of an LCA allows for a more comprehensive sustainability assessment of territories by putting into perspective the benefits offered with the impacts generated by the multifunctional nature of territories via the eco-efficiency ratio of T-LCA.

#### **5.03.P-We315 A Participatory Life Cycle Sustainability Assessment Framework for the Appraisal of Estate Regeneration Schemes in London**

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To enable sustainable growth, the assessment and analysis of the regeneration schemes should consider their socio-economic as well as their environmental impacts. The methodology which includes the three sustainable development pillars is called Life Cycle Sustainability Assessment (LCSA). To reflect the priorities of an array of stakeholders in options appraisal, key stakeholders should be engaged in different stages of decision analysis. This study aims to develop a participatory approach to LCSA for the appraisal of estates' regeneration schemes. The complex mixed methods research design consists of a pilot study, surveys, knowledge mobility, co-design workshops, ethnographic observations, semi-structured interview, and simple mathematical calculations. Review of the literature and findings of the pilot research have assisted in identifying a preliminary list of sustainability indicators for the framework. Key stakeholders including the community members and built environment specialists have participated in a survey which have resulted in eliciting the weights (preferences) of meta criteria for the framework. The proposed framework consists of 5 stages: Goal and Scope Definition; Scenario Development; Assessment; Aggregation; Interpretation. These stages can be repeated until consensus is achieved at different phases of the project. The collaborative methods of this research are expected to assist in developing a framework for the assessment and analysis of the lifecycle sustainability of regeneration schemes to be used for consensus and holistic decision-making on the appraisal of housing estates in London. The proposed framework benefits from taking into account the priorities of the key stakeholders of the schemes, and using simple and transparent mathematical calculations for interpretation of the assessment results to make it suitable for non-expert stakeholder groups. The study aims to apply its findings to be implemented to support better decision-making on the regeneration of housing estates and other types of regeneration projects in different locations.

#### **5.03.P-We320 Simulating Sustainable Farming Strategies in Luxembourg with a Hybrid Agent-Based and Life-Cycle Assessment Model**

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Agent-based modelling (ABM) has been gaining interest in socio-economic systems modelling since it allows to consider heterogenous agents and their interactions. This is particularly relevant in agricultural systems since the farms are run by actors (often family businesses) which interact with each other and whose strategies can be affected by personal preferences and

traditions and not only by profit maximization. To understand to what extent environmentally sounder agricultural and farming practices can be enforced and how these can spread among farmers, we implemented a hybrid ABM-LCA model and used it to simulate and compare different sustainability-driven scenarios.

We simulated five different scenarios. Scenario A is the baseline scenario, which represents the current situation. Scenario B considers reducing the stocking rate from 1.6 to 1.3 livestock unit per ha (LSU/ha). Scenario C brings the stocking rate further down (to 1 LSU/ha). Scenario D aims at reducing the soybean ratio in animals' feed rations to the minimum possible level that is feasible for each farm. Scenario E simulates an increase in soybean autonomy, i.e. the farmers with the highest green consciousness choose to use local soybean (instead of imported one), increasing its cultivation. The LCIA scores are calculated automatically in Python using the ReCiPe LCIA method in the Brightway2 LCA framework and relying on the *ecoinvent 3.7.1* database for the background processes.

As expected, when we reduce the stocking rates all impact categories show improvement. In fact, as there are fewer and fewer animals in the system, freshwater ecotoxicity and eutrophication categories show significant improvements since less manure is produced and thus there is less nitrogen leakage to the soil. Globally, Scenario C scores the best in almost every category. However, enforcing this scenario involves a reduction in farmers' revenue, that would have to be compensated by subsidies to make it accepted by farmers.

We also quantified the *systemic uncertainty* of the model, i.e. the uncertainty due to the inherent variance of the underlying system. The results show that the highest variability is obtained for the Resources endpoint categories.

In conclusion, the simulation tool we built can help policymakers to simulate possible emission mitigation strategies and quantify the subsidies that should be put in place to convince farmers to follow more environmentally sustainable practices.

### 5.03.P-We325 Weighted Eco-efficiency for Energy Systems: What Can We Learn From Different Stakeholder Groups?

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Nowadays energy systems are expected to comply with a great range of technical, economical, environmental, and social criteria. Due to the pressuring climate targets launched by the European Union, more attention and effort is paid to decarbonize the current energy systems, e.g. by integrating more renewable energy carriers into the electricity grid.

Next to economic evaluations of energy systems, more environmental assessments were established over the last decade.

However, decision-makers and stakeholders are left with a wide range of indicators to find consensus amongst the evaluated energy systems. Additionally, stakeholders might express different preferences for the single indicators.

Therefore, a new method is proposed to reflect the stakeholder preference for economic and environmental evaluation of energy systems. To exemplify this new method and the results of different stakeholders, a case study of a residential energy system is presented.

First, the energy system is designed and optimized using mixed-integer linear programming. Second, the carbon footprint (CF) and the levelized cost of electricity (LCOE) are calculated and integrated into the model. Third, the CF and LCOE are then weighted according to the preferences of different stakeholders. Consumption and generation data of the residential energy system originate from primary sources. Weighting data are thereby obtained from stakeholder workshops. Forth, the weighted CF and LCOE are used to calculate the eco-efficiency of the energy system for various stakeholders. Fifth, in the same manner, the eco-efficiency of the national grid is determined and used as a benchmark.

Overall, a higher eco-efficiency will represent higher emissions and costs. Therefore, the eco-efficiency of energy systems integrating more renewable energy sources will be expected to be lower, compared to the benchmark.

In general, this study exemplifies the linkage of CF and LCOE methodology with energy system programs, which can be adapted to other sites. Besides, the variance of the eco-efficiency of different stakeholders is presented.

In the context of the European energy crisis, the proposed method provides a valuable assessment for further accommodating renewable energy carriers.

### 5.03.B LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Comprehensive Analysis

#### 5.03.B.T-01 On the Complexity of Sustainable Production-consumption Systems: Using Agent-based Modelling to Understand the Role of Sustainable Behaviours

**Gustavo Larrea Sr**, **Enrico Benetto**, **Antonino Marvuglia** and **Tomas Navarrete Gutierrez**, Luxembourg Institute of Science and Technology (LIST), Luxembourg

The effect of sustainable behaviours across supply chains is important to understand key factors and potential intervention opportunities both at supply and demand level. However, for the suppliers that adopt a “sustainable” behaviour there is no guarantee that this will result also in an economically profitable strategy. Thus, the need to investigate beforehand, in a simulation environment, the consequences of this behavioural shift, both from the perspective of the single supplier and of the entire network of companies in the supply chain. For this, our objective was to answer two questions: 1) are companies always disadvantaged when adopting a sustainability-driven behaviours in a system dominated by profit-driven agents? and, 2) how much the system improves (added value, environmental impact reduction) from this behavioral change? For this, a supply network composed by producers, traders and consumers was simulated using an agent-based model where agents were programmed relying on an Algebraic Framework for Instantiating Computational Agents (AFRICA), developed for this purpose. The simulation environment was implemented using the package *pacha*, a python toolkit designed for simulating interactions of socio-technical systems. We set a pool of 202 agents composed by 100 fish-meal producers, 100 fish-meal consumers, and two wholesalers. The production and consumption networks were initialized using the Barabasi-Albert and the Watts-Strogatz models, respectively. We

demonstrated that there exist strategies and network configurations where the adoption of an environmental business norm can reduce considerably the impact of the system whilst not representing a risky decision for the agent. Moreover, when a sustainability-driven company appears randomly in the network, the probability of going bankrupt does not vary significantly even when the system is dominated by profit-driven agents. In contrast, when the green companies appear in locations closer to the consumption side, the risk for the agent is even lower and the increase in environmental performance is much higher. The utility of our study is twofold. First, a clear notion of the risk of adopting a particular business norm can facilitate companies' decision with respect to becoming an AOC. And secondly, understanding the effects that individual changes produce over the system and over the agents can aid the design of more adequate policies.

### **5.03.B.T-02 Life Cycle Sustainability Assessment using the Social Footprint Methodology 2021: Comparing Sustainability Performance of Bio Fiber with Conventional Glass Fiber**

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Life cycle assessments are frequently used by organizations to evaluate and compare the environmental performance of their processes, even though it is well known that sustainability goes beyond environmental performance and that the social and economic performance of products is also particularly important.

One cause for this is the lack of a standardized method or indicator for evaluating the sustainability performance of a good or service that includes all the effects linked to its social, environmental, and economic performance.

Weidema provides an open-access methodology called "Life Cycle Sustainability Assessment using the Social Footprint methodology 2021" to overcome this challenge. It provides a summary measure of income redistribution and all productivity-decreasing externalities associated with a particular product or activity. This model applies sustainable well-being (utility) measured in Quality-Adjusted person-Life-Years (QALY) as a summary indicator for all social, ecosystem, and economic impacts. By having a cardinal-scale quantitative indicator, QALY, this method helps to quantify trade-offs and synergies between impact categories and compare business decisions, performance, and improvement options across the industry sector.

This study demonstrates the model's potential by calculating the total QALY value for hemp and glass preform from cradle-to-grave to compare their sustainability while taking into account all the impacts associated with the social, ecosystem, and economic aspects.

Additionally, all impacts were included in the pricing, and the real value of the preforms was determined in euros. The results show that when the impacts were internalized, the price of hemp and glass fiber increased. In addition, strengthening the non-production-specific impact (impacts of missing governance), specifically addressing the labor costs of the farmers, is essential to improve the sustainability impact of hemp fibre production.

In the end, the contributions of particular impact pathways and their indicators scores were defined. The total impact category impact for the hemp and glass preform for each UN Sustainable Development Goal (SDG) and area of protection draw a clear direction to act upon.

### **5.03.B.T-03 Implementing and Extending Different Types Of LCA, Focusing on Sustainability Assessment of Investment Products**

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There are various types of Life Cycle Assessment (LCA) methods to assess the environmental impact of product systems, especially when considering an open framework. In this study, we review LCA types and in a second step investigate these specifically in the context of investment products. We focus on two prominent types of attributional LCA (ALCA) and consequential LCA (CLCA), as (1) they answer different relevant ethical research questions and (2) are prominently present in literature with many databases considerably aligning to either one of them. Within the context of investment products, EU regulations and investor demand pushing the investment industry towards life cycle or supply chain assessment of investment products, applying LCA to investment products is the obvious solution, but the choice of method and dataset are not straightforward for identifying the investment product with the lowest environmental impact per euro. We will tackle specific challenges for this context: (1) investments directly or indirectly lead to effects on production and consumption through financial, economic and corporate processes, (2) investments may lead to different sets of products, which should be considered, and (3) there are also different types of investment products (e.g. debt versus equity). For the first challenge, we consider these mechanisms as a black box and look at the finality of effects. For example, in case of a product amount increase, the ratio of product amount per euro needs to be derived. Concerning the second challenge, product utility needs to be regarded to have an adequate comparison in ALCA. For CLCA, this is not always needed. Most importantly, when considering a demand-constrained market, producing more will just shift the market composition. In other words, a substituted market mix that provides the same functionality as that of the product, will go down in tandem. For the different investment types, we propose specific types of LCA, e.g. organizational ALCA (e.g. using IO-based databases) in case of stocks based on the company share, this for an attributional perspective. The study is timely, as it serves as a guide for applying LCA to investment products, in line with upcoming regulations and investor demand. A more thorough analysis and case study are being finalized.

### **5.03.B.T-04 Methodological Approach to Account for Natural Hazards in the Life Cycle Assessment of the Energy Production Sector**

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Several factors must be considered when addressing planning and decision-making for the present and future of the electricity production sector, such as logistics related to the geographic location, and technical, social, political, economic, and potential environmental aspects. The latter can be estimated through environmental impact assessments. Life Cycle Assessment (LCA) is one of the widely accepted tools to obtain relevant results for planning and decision-making.

Despite the global scope of the LCA method, its typical application focuses on evaluating scenarios describing average or steady states, thus not accounting for the potential environmental consequences of abnormal events, such as natural hazards that can result in important damages. However, it is imperative to consider the latter in the environmental assessment since their possible consequences may irreversibly change the planned projects negatively for all stakeholders.

A way to approach this is to take a scenario-based and parametrized approach to LCA. It aids the practitioner in considering multiple situations in the product system, but this has limitations in a sector-wide evaluation that considers the negative influences of natural hazards on the system and the environmental impacts of said disturbances.

The purpose of this work is to address these limitations by analysing each phase of the LCA methodology and adapt it for a comprehensive treatment of natural hazards in LCA. The objective is to provide a decision-making framework that supports decision-makers in making decisions based on the knowledge and needs of the present that will still be considered fair decisions in the future, especially when considering impacts related to the occurrence of a significant natural event.

As a first attempt, the analysis and the methodological approach to design are based on a case study on the France's photovoltaic (PV) pathway. Only a few natural hazards, known to be able to cause important disturbances and damages to PV systems are considered. The results from the case study will not be significant to represent a real situation and draw relevant conclusions within the PV sector. However, they will illustrate the strengths, limitations, and feasibility of the proposed methodological framework, thus permitting its continuous improvement and open potential scientific discussions and developments for the PV and the electricity sector.

### **5.03.B.T-05 Towards Prospective Territorial LCAs: Considering the Effects of Global and Local Environmental Changes on the Performance of Irrigated Areas**

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Territorial Life Cycle Assessment (T-LCA) is a meso-scale adaptation of the conventional LCA framework, allowing to assess the environmental performance of a territory and an associated land planning scenario while considering its multifunctionality (e.g. economic, social or environmental land use functions). For that purpose, eco-efficiency ratios, i.e. services provided by the territory to its environmental impacts, are computed. To date, the approach has been static, assessing the eco-efficiency of a scenario for a given year. However, the environmental performance of a system can be affected by feedback from the ecosphere, which are stimulus on the ecosphere that are returned to the technosphere through a series of interactions. This issue is particularly important to determine 'no-regret' land planning scenarios over the long term.

This study aims at developing a methodology to assess both global and local environmental feedback on the environmental performance of scenarios. The proposed methodological developments will be used to compare the evolutions of eco-efficiencies of agricultural land planning scenarios with and without irrigation considering the effects and interactions of climate change and water scarcity over time.

The core of the methodology is based on a coupling between a crop model, the Aquacrop model of the Food and Agriculture Organization of the United Nations (FAO), and the T-LCA approach. The first step consists in defining the land planning scenarios to assess, i.e. in this study, agricultural planning scenarios using different crops with the implementation or not of a hydraulic infrastructure to secure local water supply (i.e. a hill reservoir). The next step is data collection, i.e. input data for Aquacrop and data for the Life Cycle Inventory (LCI). Third, climate change and water scarcity feedback are taken into account through modelling with Aquacrop. Fourth, LCI are built with primary data from Aquacrop simulations, i.e. yields and water balance, and secondary data from existing LCA database, literature and expertise. Finally, the environmental performance of the scenarios are compared with their eco-efficiency ratios, calculated with the mass of agricultural products (one of the services provided by the territory).

These works highlight the importance of considering global and local environmental changes when assessing the environmental performance of long term land planning scenarios to make "no regret" decisions.

### **5.03.P LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Comprehensive Analysis**

#### **5.03.P-We315 A Participatory Life Cycle Sustainability Assessment Framework for the Appraisal of Estate Regeneration Schemes in London**

*Sahar Nava<sup>1</sup>, Esfandiar Burman<sup>2</sup> and Zaid Chalabi<sup>2</sup>, (1)Bartlett School of Environment, Energy and Resources, UCL, London, United Kingdom, (2)Bartlett School of Environment, Energy and Resources, University College London, United Kingdom*

To enable sustainable growth, the assessment and analysis of the regeneration schemes should consider their socio-economic as well as their environmental impacts. The methodology which includes the three sustainable development pillars is called Life Cycle Sustainability Assessment (LCSA). To reflect the priorities of an array of stakeholders in options appraisal, key stakeholders should be engaged in different stages of decision analysis. This study aims to develop a participatory approach to LCSA for the appraisal of estates' regeneration schemes. The complex mixed methods research design consists of a pilot study, surveys, knowledge mobility, co-design workshops, ethnographic observations, semi-structured interview, and simple mathematical calculations. Review of the literature and findings of the pilot research have assisted in identifying a preliminary list

of sustainability indicators for the framework. Key stakeholders including the community members and built environment specialists have participated in a survey which have resulted in eliciting the weights (preferences) of meta criteria for the framework. The proposed framework consists of 5 stages: Goal and Scope Definition; Scenario Development; Assessment; Aggregation; Interpretation. These stages can be repeated until consensus is achieved at different phases of the project. The collaborative methods of this research are expected to assist in developing a framework for the assessment and analysis of the lifecycle sustainability of regeneration schemes to be used for consensus and holistic decision-making on the appraisal of housing estates in London. The proposed framework benefits from taking into account the priorities of the key stakeholders of the schemes, and using simple and transparent mathematical calculations for interpretation of the assessment results to make it suitable for non-expert stakeholder groups. The study aims to apply its findings to be implemented to support better decision-making on the regeneration of housing estates and other types of regeneration projects in different locations.

### 5.03.P-We316 (How) Can Ontologies Help to Integrate Sustainability Dimensions? Learnings From the ORIENTING Project

**Thomas Sonderegger<sup>1</sup>**, **Chris Mutel<sup>1</sup>**, **Till Bachmann<sup>2</sup>**, **Luca Zampori<sup>3</sup>** and **Janire Clavell<sup>4</sup>**, (1)*Ecoinvent Association, Switzerland*, (2)*EIFER, Germany*, (3)*PRé Sustainability, Netherlands*, (4)*Tecnalia, Spain*

ORIENTING is a H2020 funded project, which aims at integrating different sustainability topics –the environmental, economic, and social domains as well as material circularity and criticality assessments – into a single operational methodology for Life Cycle Sustainability Assessment (LCSA). This needs alignment of concepts and data structures across the different topics for which ontologies were considered helpful. An ontology is a formal description of concepts and their relationships in a domain. For example, activities (or processes) and flows (or exchanges) are concepts in the LC(S)A domain, related by flows being inputs and outputs of activities.

ORIENT builds on the BONSAI ontology which itself builds on previous ontologies suggested for sustainability assessments. To capture all ORIENTING topics, the BONSAI ontology needed extensions. This meant adding new classes, sub-classes, and ranges of instances to a) capture missing concepts such as stakeholders in social LCA; b) further distinguish existing elements (such as flows) into different types; and c) introduce LCIA.

ORIENT was used in ORIENTING as guidance to, for example, assign differently named data points used in the different sustainability topics to the same or equivalent classes. This was achieved by an ontology visualisation, which was discussed with topic experts. In a similar way, ORIENT could guide discussions in the LC(S)A community about, for example, data formats and conceptual and terminological alignment. A technical implementation would allow a connection to data and help in data integration as exemplary shown by the BONSAI ontology authors. This was beyond the scope of ORIENTING and would come with a considerable amount of technical expertise needed. This leaves us with several trade-offs and related questions about further use of ontologies in the LCSA field: Visualization only versus full technical implementation: To what degree can and should ontologies be used in the LC(S)A field? A working ontology offers a connection to the semantic web and data in it: Which and how much semantically classified data is available? Are there other (and maybe easier) ways to achieve our goals such as data collection and integration? An ontology could be at the core of data format converters: Is this feasible or does every conversion have its own rules?

With this contribution, we would like to stimulate a discussion based on the experiences made in ORIENTING.

### 5.03.P-We317 LCSA and Sustainability

**Tomas Ekvall**, *TERRA, Sweden; Technology Management and Economics, Chalmers University of Technology, Sweden*

Life cycle sustainability assessment (LCSA) is often defined as the sum of life cycle assessment (LCA), social LCA, and life cycle costing (LCC). Both LCA and LCC calculate impacts per functional unit – typically a basic unit chosen to quantify the function generated by the product or service investigated (e.g., supplying a litre of oil, a cubic meter of gas, or a MJ of heat). The results can be used to measure environmental and economic efficiency, i.e., what volume of functional output we can get for a given environmental or economic cost. However, efficiency is just one perspective on sustainability. Environmental sustainability depends less on the environmental efficiency and more on the total environmental impacts. A gas pipeline might deliver gas with less climate impact per cubic meter, compared to the shipping of liquid natural gas; if the pipeline makes the gas more accessible and cheaper, however, it can still increase the climate impact by stimulating an increased use of the fossil gas. By changing the calculation basis from the basic unit of the functional output (e.g., a cubic meter of gas) to a time span (e.g., 1 year) or to a specific project (e.g., a pipeline), the LCSA can account for changes in the functional output and generate a more relevant measure of environmental sustainability. The equivalent approach in LCC means presenting the total cost for the time span or project rather than the cost per litre or MJ. However, economic sustainability depends not only on projected costs, but also on economic risks and business opportunities, and on the existence of applicable market and business models. The Nordstream gas pipelines reduced economic risk by adding new routes for transferring gas from Russia to the EU, but increased economic risk by reinforcing the dependency of EU on Russian gas. Such important aspects of economic sustainability can typically not be quantified. Hence, a sustainability assessment should include also qualitative aspects. The impact aspects of social sustainability are highly case-specific. For the Keystone XL pipeline, the crucial social aspect was the ground over which the pipeline would have run. The Nordstream and Keystone cases illustrate that key sustainability aspects need not have a cradle-to-grave perspective. Hence, to assess the sustainability of different options an LCSA should have a broad systems perspective but need not have a cradle-to-grave perspective in all parts.

### 5.03.P-We318 Investigating Switching Shipping's Fossil Fuel Use with Alternative Options for a Low Carbon Future - A Comparative LCA Study



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The international shipping sector is responsible for 3% of total global GHG emissions, and is in urgent need of decarbonisation. Alternative fuels are a potential solution, therefore, this research compares the environmental burden of a broad range of fuel options including: Hydrogen (green and blue), Ammonia (green and blue), E-methanol, bio-methanol, bio-methane, and FAME biodiesel. Each potential option has varied life cycle GHG contributions due to different production methods and on-board use. Life cycle assessment (LCA) techniques are applied to compare each fuels' potential to lead the sector towards a sustainable, low carbon transition. GaBi software and ReCiPe2016 are utilised alongside EcoInvent 3.7 and further data from literature.

The research presented establishes novel inventories for the production and use of alternative fuels on board shipping vessels. A top down analysis of the sector's global propulsion energy is performed and combined with LCA results to investigate each alternative fuels' capacity for global shipping GHG emission reduction. Consideration is given to alternative fuel production capacities, both now and in the future, as well as to the shipping industry's relationship with the wider energy system.

The results reflect the complexity of the challenge at hand. For example, GHG emission reduction from non-biogenic Methanol is found to be limited, due to its carbon content. On the other hand, restricted capacity for sustainable production of bioenergy feedstocks limits the extent by which the biogenic carbon cycle can be utilised for GHG emission reduction. This research also found that nitrous oxide from ammonia combustion could result in Ammonia fuel having significantly higher GHG emission potential than previously expected by literature.

Overall, GHG reduction is shown to be possible with a switch to alternative fuels, provided that the sector has a commitment to a life cycle perspective on decarbonisation. There is also a requirement for the timely development of global production capacity of alternative fuels, and the introduction of energy efficiency measures within the industry if decarbonisation is to be achieved. This research offers contributions to LCA inventories for alternative fuel production and use, and advances understanding of methods to account for shipping's global scale within LCA studies.

### **5.03.P-We319 Pioneering Historical LCA - A Perspective of the Development of Personal Carbon Metabolism 1860 to 2020**

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The world at large has agreed that anthropogenic climate change is an urgent matter, that should be mitigated. Different schools of thought exist on how to reduce the emission of greenhouse gases, a debate sometimes referred to as "boomsters vs doomsters", arguing whether or not technological change inevitably will solve the issues, or if we humans have to put more effort in changing our consumption patterns, as to achieve a sufficient green transition. The IPAT equation conceptually defines the environmental impact (I) to be a product of the population (P), its' affluence (A), and the technological efficiency (T), and can hence give a perspective to the debate.

A Danish research project investigates the personal carbon metabolism of Danish urban citizens, and how it developed since "the black transition" which occurred at the industrial revolution almost 200 years ago. The overarching objective is to develop scientifically backed museum exhibitions, which can provide a public debate and perspective on the current green transition. The project is an interdisciplinary collaboration between museums and researchers within both humanities and engineering.

The personal carbon metabolism is quantified via historical LCA – an until now unexplored field. This LCA can be considered partially dynamic, and has similarities to the field of prospective LCA. The LCA considers changes in consumption as well as technology, and includes a range of significant consumption segments; namely transport, food, housing, and energy use. By estimating the LCA at five timesteps, a perspective is provided on the personal carbon metabolism and how it has changed in the period 1860-2020.

Due to the historic nature, data availability is a significant challenge, hence different means to provide qualified scenarios are explored. The project is ongoing till spring 2023, but current results reveal that the massively increased technological efficiency is not sufficient to keep up with our even more drastically increased affluence. On the more bright side however, the results indicate that the focus on the green transition has led to a decrease in the accelerating growth since the 1970's. The findings of the research is of relevance as it provides a historical perspective on how changes in technology and consumption has affected the impact on climate change, empowering citizens and scientists to see the current green transition in this context.

### **5.03.P-We320 Simulating sUustainable Farming Strategies in Luxembourg with a Hybrid Agent-Based and Life-Cycle Assessment Model**

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Agent-based modelling (ABM) has been gaining interest in socio-economic systems modelling since it allows to consider heterogeneous agents and their interactions. This is particularly relevant in agricultural systems since the farms are run by actors (often family businesses) which interact with each other and whose strategies can be affected by personal preferences and traditions and not only by profit maximization. To understand to what extent environmentally sounder agricultural and farming practices can be enforced and how these can spread among farmers, we implemented a hybrid ABM-LCA model and used it to simulate and compare different sustainability driven scenarios.

We simulated five different scenarios. Scenario A is the baseline scenario, which represents the current situation. Scenario B considers reducing the stocking rate from 1.6 to 1.3 livestock unit per ha (LSU/ha). Scenario C brings the stocking rate further down (to 1 LSU/ha). Scenario D aims at reducing the soybean ratio in animals' feed rations to the minimum possible level that is feasible for each farm. Scenario E simulates an increase in soybean autonomy, i.e. the farmers with the highest green consciousness choose to use local soybean (instead of imported one), increasing its cultivation. The LCIA scores are calculated automatically in Python using the ReCiPe LCIA method in the Brightway2 LCA framework and relying on the *ecoinvent 3.7.1* database for the background processes.

As expected, when we reduce the stocking rates all impact categories show improvement. In fact, as there are fewer and fewer animals in the system, freshwater ecotoxicity and eutrophication categories show significant improvements since less manure is produced and thus there is less nitrogen leakage to the soil. Globally, Scenario C scores the best in almost every category. However, enforcing this scenario involves a reduction in farmers' revenue, that would have to be compensated by subsidies to make it accepted by farmers.

We also quantified the *systemic uncertainty* of the model, i.e., the uncertainty due to the inherent variance of the underlying system. The results show that the highest variability is obtained for the Resources endpoint categories.

In conclusion, the simulation tool we built can help policymakers to simulate possible emission mitigation strategies and quantify the subsidies that should be put in place to convince farmers to follow more environmentally sustainable practices.

### **5.03.P-We321 Sustainable Freshwater Consumption of the Agricultural Crop Production in a Highly Dense Population Setting**

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Global crop production consumes a huge amount of freshwater due to which regional freshwater overconsumption is evident from major watersheds of the world. We as a global community are facing the dilemma of identifying ways to feed the billions of people and ensuring that our food production follows a sustainable practice. Bangladesh is a densely population country with one of the lowest per capita agricultural lands of the world. In recent days, the country is gradually becoming self-reliant on food production. There are few issues regarding the limited freshwater resources of the country: seasonal freshwater overconsumption during the dry period, and water pollution due to agricultural intensification. In this study, we aim to assess the sustainability of the crop water use for supporting Bangladesh's diet and gain the implications for future sustainable national diet focusing on the aspect of water resources. The updated WaterGAP 2.2d model is used to calculate the freshwater overconsumption of Bangladesh's watershed, and the overconsumption associated with the crop production in the country is estimated corresponding to the total agricultural production for 1960–2019 period using updated crop evapotranspiration value. According to the results, the average overconsumption of freshwater from agricultural crop production during 2000–2016 period in Bangladesh was ~20,433 million cubic meter which was mainly due to own consumption of several crops e.g., rice, areca nuts, wheat, jute etc. Furthermore, we also seek to identify the relationship between the nutrient density of the food crops and induced freshwater overconsumption, which gives the implications towards the achievement of the sustainable national diet. The outcome from this study will provide strategies for sustainable crop production in the country with the identification of the key crops and the watersheds that need improvement in the context of sustainability of water use.

### **5.03.P-We322 Environmental Impacts and Sustainability Implications of Aquaponics-exploring Aquafeed**

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Closed loop agricultural systems, such as aquaponics, have the potential to reduce the environmental impact of food production through the efficient usage of nutrients and water. Aquafeed, however, with critical components such as fish meal and fish oil often harvested from wild forage fish population are a growing area of concern which has led to a number of potential replacements being considered. Not all replacements have a lower environmental impact, as it is dependent as to what materials they are sourced from. Life cycle assessment and multicriteria decision analysis informed by farmer survey data will be used to explore this issue. A midpoint life cycle assessment using the TRACI environmental impact categories will be conducted along with survey data gathered from aquafarmers in Wisconsin, USA. This work found that although some fish meal and oil replacements in aquafeed have a lesser environmental impact than conventional feeds, that is not true across the board. Further, aquafarmers are heterogenous in their beliefs which influence the potential for them to adopt fish meal and oil free feeds. This study is particularly relevant as aquaponics and aquaculture are fast growing methods of food production, and current forage fishing practices have the potential decimate food webs.

### **5.03.P-We323 Economic and Environmental Assessment of Two Industrial Symbiosis Opportunities in the Copper Sector**

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Sustainability is a multi-faced topic, and industry has acknowledged the need to pursue it for increasing its resilience. One way to do this is via Industrial Symbiosis (IS). IS is the exchange of by-products among different stakeholders, as well as the sharing of infrastructure and the joint provision of services to increase material and energy efficiency and achieve cost savings. One of the sectors with big amounts of by-products and high impact is the copper sector. Looking at opportunities for IS and measuring its benefits is a way to improve the sector's footprint. In this project, we first analysed which Cu by-products and Cu-bearing

products would be eligible for IS. The streams viable for IS were identified by means of a literature search. The potential economic and environmental benefits were quantitatively assessed using a market study/cost-benefit analysis and an LCA, respectively, and were combined to build a business case. Two relevant material streams were identified: iron silicate (Fe-Si) from Cu slags from smelting and refining, and End-of-Life Electric Vehicle Batteries (EOL EVB). The IS scenarios built were the use of Fe-Si to replace natural aggregates in cement and concrete manufacturing or in road construction, and the use of EOL EVB in large scale energy storage applications, replacing new batteries using virgin materials. The results of the LCA showed that, in the case of Fe-Si, the impacts of IS are significantly lower (> factor 2) compared to using gravel in roads. When natural aggregates are replaced by Fe-Si in cement and concrete production, the impact is also lower. For EOL EVB, the IS scenario scored better in most of the impact categories, with a 28% improvement in climate change, 23% in resource use of minerals and metals, and 48% in resource use of fossils. Most of the environmental benefits were found to come from avoiding the production of new batteries from virgin materials. The market analysis showed a good opportunity for stakeholders in the sector in both cases, as demand for raw materials is increasing. The cost analysis was promising for Fe-Si, with a case-by-case study needed, considering local context. For EOL EVB, re-purposing was found to be cheaper than recycling and reuse cheaper than new battery use. The study has shown that IS can be economically feasible and environmentally beneficial to the copper sector and can create an opportunity to decrease the sector's footprint and increase its circularity.

### **5.03.P-We324 Understanding What Is Required To Make Reusable Packaging Sustainable: Combining Life Cycle Assessment and People's Willingness**

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Today plastic pollution has become one of the most pressing environmental issues. Plastic packaging can be particularly problematic as it accounts for 50% in weight of the total plastic waste in the world and it is a linear system; raw materials are taken from the Earth to make packaging products and then, after being used by consumers, thrown away as waste. Therefore, to develop a more sustainable plastic packaging system, the focus should be on new design and technology to move to a more circular economy and keep materials in use to prevent waste and protect and restore the environment. One of the solutions to change from a linear system to circular is the reuse system model. However, there is currently limited understanding about required factors to implement such a circular economy. While the reuse system should be environmentally beneficial, it needs to be accepted by people to be implemented in practice not only be technically possible or possible in theory.

This presentation will discuss work from the "Many Happy Returns" project that examines the sustainable reuse of plastic packaging by identifying the best reuse model for different contexts, exploring the optimum materials, process and technologies for smart reusable plastic packaging systems and developing practices to encourage reuse. People's willingness was measured and integrated into LCA results to show how technology and packaging design can make a balance between these to enable a reuse system as a sustainable solution for plastic waste. In particular, analysis of returnable cups and bowls for use in cafes as take-out packaging, were analyzed. The analyzed midpoint impact categories have been aggregated into the three main life cycle phases: production, use and End of Life (EoL). Then, they have been used to assess the environmental break-even point (BEP), i.e. the minimum number of uses necessary for a returnable cup and bowls to be preferable than some single-use ones. Results showed that the weight of bowls, moving to renewable energy and End of Life (EoL) of the reusable products are key factors to identify possible strategies to improve the implementation and success of reusable products.

### **5.03.P-We326 Life Cycle Assessment of Room Air Conditioners Using Big Data of 70,000 Units in Japan**

**Genta Sugiyama<sup>1</sup>, Tomonori Honda<sup>2</sup> and Norihiro Itsubo<sup>1</sup>**, (1)Tokyo City University, Japan, (2)National Institute of Advanced Industrial Science and Technology, Japan

It is estimated that two-thirds of the households in the world will have air conditioners installed, and the current energy demand for air conditioning, which accounts for 10 percent of global electricity consumption, will be tripled by 2050. Economic growth in developing countries has allowed their residents who could not have air conditioners installed in their homes to do so. Global warming also increases the demand for air conditioners. The increased demand for air conditioners burdens the electricity systems in many countries as well as boosting the amount of greenhouse gas (GHG) emissions.

In Japan, about 25 percent of the entire residential GHG emissions comes from the use of air conditioners. The country has set a goal to reduce GHG emissions by 66 percent from the 2013 level to achieve carbon neutrality by 2050. One of the problems arises, however, regarding the calculation of assessments, because the data that shows the use of air conditioners in the Tokyo area is taken as a standard value to indicate the data of the entire Japan. Since the Japanese islands stretch from north to south, there is a concern that the assessments may be inaccurate disregarding a variety of diverse in Japan.

In this study, I have looked into big data, i.e., the data that describes actual usage of about 70,000 air conditioners in Japan from 2017 to 2021 to illustrate the discrepancy between actual data and the standard values. I also conducted a life cycle assessment reflecting the actual usage. I have found that the actual averages are about 18% lower in annual operating hours and 23% lower in annual energy consumption than the standard values, thus the assumption of the standard values may be overestimated. This divergence resulted in a variation of approximately 28% (22%~37%) in the LCA results for air conditioners. In my future study, I will estimate future heating and cooling demand associated with future temperature changes based on actual usage by region, and make proposals for air conditioner usage methods to achieve carbon neutrality.

### **5.03.P-We327 Sustainability Assessment of Plasma Gasification as Waste-to-energy Strategy: Review on Environmental, Economic, and Social Aspects**

**Ana Isabel Ramos, INEGI, Portugal**

Following the actual pace of industrial development, due to the ever-growing tech- and resource-savvy society, unprecedented waste amounts are generated on a daily basis. Therefore, waste-to-energy (WtE) techniques appear as a possible approach to convert solid residues into energy, within more energy-efficient processes to emit less or no CO<sub>2</sub>. Plasma gasification constitutes a proficient and clean alternative with multiple environmental gains, simultaneously reducing or eliminating toxicity and pollution issues while avoiding the landfilling of huge amounts of residues. To evaluate the performance of this WtE treatment, the life cycle thinking (LCT) methodology gathers useful impact assessment tools, providing quantifiable indicators on multiple domains. The lack of works reporting the combined assessment of the environmental, technical, economic and social spheres of WtE has been previously noticed and, to the best of the authors' knowledge, there is no such review so far for plasma gasification. This work proposes a deep review of literature on the life cycle assessment, life cycle costing and social impact assessment of plasma gasification, to evaluate how LCT tools are applied to the energetic valorization of solid residues by this technique, as a measure of sustainability. This provides insights on the contribution of energy recovery as a circular strategy, diverting waste from open dumps or landfills, while producing renewable energy and/or by-products that will replace the use of virgin materials. Herein, an attempt to complement the knowledge in the sectors of renewable energies and waste management is conducted, to support the fulfillment of circular economy goals and contribute to a more sustainable society and way of living.

Keywords: plasma, gasification, LCA, LCC, social assessment, circular economy

### **5.03.P-We328 Sustainability Risk Screening: The Definition and Undertaking of a Counterfactual Sustainability Screening Assessment**

**Edward George Platt<sup>1</sup> and Peter Styring<sup>2</sup>, (1)The Department of Chemical and Biological Engineering, University of Sheffield, United Kingdom, (2)Chemical and Biological Engineering, University of Sheffield, United Kingdom**

The need for screening sustainability assessments, especially for low Technology-Readiness Level (TRL) technologies, is at its highest point given the extreme rate of innovation as the chemical synthesis industry looks to source sustainable supply chains. A screening assessment allows for the early understanding of the sustainability of low-TRL technologies based on scaled or simulated data, allowing for the detection of "hotspots" but rarely as a tool for informed decision-making. This research looks to define and prove, as a concept, a counterfactual sustainability screening assessment which looks to assess the sustainability performance of low-TRL technologies compared to the current, fossil-based industry standard. The method employs a frequency-based, semi-quantitative assessment methodology similar to that of a standard risk assessment. For a list of environmental, economic and social metrics, the likelihood of a process' performance outperforming the current industry standard allows for the generation of a single score, without the need for exact quantitative impacts for each metric. The decision to pursue a sustainability risk assessment is down to the accepted idea that predicting the probability of a process' sustainability impact exceeding a defined benchmark avoids many of the subjectivity pitfalls associated with predicting the exact impact of a process' adoption, a concept outlined by Glenn Suter in the Journal of Environmental Management. This will therefore likely reduce variance between assessment undertakers and therefore dampen the effects of subjectivity on the final result.

This presentation looks to build on previous presentations, outlining the final list of sustainability assessment metrics, followed by the definition of the counterfactual baseline and a proof of concept in the form of a sample assessment, looking at the formation of defossilised  $\alpha$ -olefins for utilisation within liquid surfactants.

### **5.03.P-We329 Assessment of Reference Values in a Social Life Cycle Assessment: Concepts, Usefulness, and Examples**

**Andreas Roesch, Melf-Hinrich Ehlers and Melanie Douziech, Agroscope, Switzerland**

The main purpose of reference values (RVs) is to facilitate interpretation of (social) indicators. RVs allow policymakers, farmers, consumers and various other stakeholders to assess better the results from indicator-based studies. Definitions of RVs are however numerous and no consensus exist either on how they should be defined. The objective of this study is therefore twofold namely first to evaluate the purpose and use of RVs, with a particular focus on social life cycle assessment in agriculture and second to provide guidance on how to derive RVs. To reach this objective, a comprehensive literature review and an internal workshop with experts from different disciplines were carried out. Three approaches were identified to derive RVs: (i) science-based values, (ii) political targets, and (iii) stakeholder/ experts opinions, whereas a clear distinction between the three is not always possible. RVs can also be derived from combining scientific findings, expert judgement, references to international conventions and statistical knowledge. From these three approaches, deriving RVs from scientifically agreed knowledge should be preferred. In fact, changing legislation, laws and rules do not allow for robust RVs definition. Another difficulty when defining RVs lies in potential conflicts when substantial economic and social impacts are anticipated, no matter whether science-based or politically determined RVs are used. This is particularly important as many agricultural issues have moved into the political limelight. In those cases, determination of references values based on compromises can be a solution. Setting objective, transparent and robust RVs to avoid the method being questioned can be an additional way of handling this challenge. The literature review further revealed that RVs are mainly used to (i) interpret indicators while allowing comparisons across space, locations, actors, companies (farms), sectors, and over time, (ii) give advice on how to improve sustainability, (iii) normalize indicators allowing subsequent aggregation and (iv) provide a reference for determining reference scales. These diverse uses suggest that how to best deal with RV would be use- and goal-specific. Future research will focus on the use of RVs in agriculture systems and on fit-for-purpose methods for deriving robust and widely accepted RVs.

### **5.03.V LCA and Beyond – Integrating Sustainability and/or Other Dimensions for a More Comprehensive Analysis**

### 5.03.V-01 Dynamic Carbon Footprint Using Factory Iot and Sensoring Systems

**Takahiro Hashimoto<sup>1</sup>**, **Norihiro Itsubo<sup>1</sup>**, **Teruo Ohashi<sup>2</sup>** and **Hiroki Ookubo<sup>1</sup>**, (1)Tokyo City University, Japan, (2)LEXER RESEARCH

According to data released by the Ministry of the Environment on greenhouse gas emissions in FY 2019, the industrial sector has the highest CO<sub>2</sub> emissions, accounting for about 35% of total domestic emissions. Since about 94% of the industrial sector consists of manufacturing, a policy shift toward decarbonization of the manufacturing sector is strongly required in order to achieve Japan's greenhouse gas reduction targets. According to the Energy Saving Factory, "A large portion of electricity consumption in factories is attributed to the production process, and in order to achieve carbon neutrality, a complete switch to renewable energy and a review of the entire production process to reduce losses and electricity consumption from existing production processes are the manufacturing industry's efforts to become carbon neutral. This is an effort for manufacturers to become carbon neutral.

In addition, while there are many existing literatures that strive to improve productivity in factories, there are few papers that evaluate "environmental impact" as one parameter, and this study, which focuses on the theme of environmental impact and productivity, is highly novel.

Based on the above, the purpose of this study is to improve productivity and reduce environmental load in a factory using IoT, simulation, and LCA, and to identify the optimal layout.

The research method is to first understand the layout of the target factory and data on energy consumption, workers, and production using data collection and sensors. In particular, for data on electricity, ammeters will be installed and accurate data at regular intervals will be obtained through Raspberry Pi. By modeling these production process information and inputting it into simulation software, the "environmental impact" and "productivity" of each production process can be visualized. By identifying production processes that have a large environmental load and low productivity from such visualized information, and repeating verification and improvement on the simulation, the maturity of factory design can be improved, and both productivity improvement and environmental load reduction can be achieved. Therefore, the optimal layout is identified and a proposal for improvement of the factory layout is proposed.

### 5.03.V-02 Exploring the Use of ProScale as a Complementary Tool in SLCA

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The research presented aimed to further assess the operationalization of the subcategory *Health and Safety* within Social Life Cycle Assessment. ProScale is a tool in development by IVL Swedish Environmental Research Institute. The tool aims to assess the toxicological potentials of product systems. Since it follows a life cycle-oriented approach, it was tried out in our Social Life Cycle assessment in an aim to explore the usefulness of ProScale to operationalize the health and safety subcategory.

Occupational health should aim at: the promotion and maintenance of the highest degree of physical, mental and social well-being of workers in all occupations the prevention amongst workers of departures from health caused by their working conditions. The results of ProScale shows that the main risk from toxicological exposure is at the spinning stage of fabric production. The main contributor is Cotton dust from the spinning process, here followed by starch and polyvinylalcohol exposure in the sizing process.

The application on ProScale as a complementary tool to the Health and Safety subcategory gave additional information on the substances included in the same lifecycle, as well as their risk of direct toxicological effect on workers in the production. The compatibility with the methodology of SLCA suggested by the UNEP Guidelines, only additional data collection of substances their massflow and H phrases was needed. ProScale is useful in the sense that the substances can be presented as a hotspot analysis comparing substances against each other. The ProScale factor has less function in indicating the general risk of the substance. A factor indicating a reference level for each substance would be a possible development for ProScale in this context. The potential of applying ProScale in similar SLCA studies is judged to be feasible and give deeper information of the product systems social risks regarding health and safety.

### 5.03.V-03 Carbon Footprint of Tourism in OECD Countries Using Satellite Accounts

**Akihiko Tsutsumi** and **Norihiro Itsubo**, Tokyo City University, Japan

The 26th Conference of the Parties to the United Nations Framework Convention on Climate Change (COP26) was held in Glasgow, Scotland, from October 31 to November 12, 2021, and the Glasgow Declaration was adopted to address climate change in the tourism industry. The Declaration calls for halving CO<sub>2</sub> emissions from the tourism industry by 2030, and virtually eliminating CO<sub>2</sub> emissions by 2050. The Declaration also includes five initiatives, one of which is to "measure and disclose all CO<sub>2</sub> emissions related to travel and tourism," and to promote LCA.

A 2017 study of Japan's tourism industry evaluated greenhouse gas (GHG) emissions and reported annual emissions of 1.36 billion t-CO<sub>2</sub>eq, with emission factors for inbound, domestic, and international travel, showing that each travel mode has its own characteristics. Although it is reported that GHG emissions from tourism account for approximately 8% of the world's total emissions, there are few examples of GHG emissions from tourism in the world.

Based on the above, the objectives of this study are (1) to compare GHG emissions in different countries, (2) to understand the characteristics of the global tourism industry, and to discuss the relationship between tourism and the environment.

This study targets inbound and domestic travel in about 50 OECD-related countries and uses the OECD Tourism Trends and Policies 2020 (OECD Data), a tourism satellite account data (hereinafter referred to as TSA) that aggregates consumption in the tourism industry, and the OECD Data, which is an economic and environmental transaction table for 190 countries around the

world. GHG emissions are calculated by combining the OECD Tourism Satellite Accounts data (hereinafter referred to as "TSA"), which aggregates tourism consumption, and the Multiregional Input-Output (MRIO) table, which is an economic and environmental transaction table for 190 countries.

GHG emissions from Japan's tourism industry in 2017 were found to be equivalent to about 5% of annual emissions. The results also show that domestic emissions are about five times higher than inbound emissions. The majority of the emissions from Japan's tourism industry are attributable to domestic travelers.

In the future, we would like to create a correlation chart based on the GDP of each country and the calculation results and discuss the relationship between the economy and the environment in the tourism industry.

### **5.03.V-04 Complementarities of Responsible Research and Innovation and Life Cycle Sustainability Assessment**

*Melanie Douziech and Melf-Hinrich Ehlers, Agroscope, Switzerland*

Responsible Research and Innovation (RRI) becomes increasingly relevant, for example in European research and innovation programs. RRI aims to guide research and innovation towards socially desirable outcomes by means of collective stewardship. Four keys facilitate this collective stewardship: anticipation, reflexivity, inclusion and responsiveness. Anticipation explores futures of relevance to identify risks and opportunities. Reflexivity implies to put assumptions to a test. Inclusion means participation of stakeholders and the wider public in discussions and actions on research and innovation. Finally, responsiveness is the ability to change direction and shape of research and innovation based on new findings. Each key can be implemented using opening-up and closing-down methods to either gather diverse perspectives on a subject or to make decisions on how to progress with a project. We explore how RRI and sustainability assessment can complement each other in attempts to achieve desirable social and environmental impacts of research and innovation projects. First, we summarized key-specific methods that support RRI. Second, we reviewed relevant sustainability assessment methods. Third, we identified key advantages and drawbacks of each approach. Finally, we explored how life cycle sustainability assessment (LCSA) – the combination of life cycle assessment (LCA), social LCA and life cycle costing – can complement RRI in terms of aims, methods and results. Besides opening-up or closing-down discussions on RRI, the methods to implement RRI keys can be useful in various steps of LCSA. They can help identifying which indicators are most relevant for project stakeholders. Knowledge of these methods can be particularly useful in generating data needed for social LCA. Quantitative results of LCSA can trigger reflexivity or be a valuable input for anticipation activities. Stakeholder participation in selecting relevant indicators for a LCSA supports the inclusion key, while outcomes of participatory deliberations on RRI consultations could trigger demand for LCSA. Building on these preliminary findings, future work will investigate possibilities to combine RRI and LCSA, or sustainability assessment more generally.

### **5.03.V-05 Life Cycle Cost-Benefit Analysis of Reinforced Concrete River Wall**

*Mony Rith So<sup>1</sup> and Norihiro Itsubo<sup>2</sup>, (1)Environmental Management, Tokyo City University, Japan, (2)Tokyo City University, Japan*

Global warming and continued development in flood-prone areas progressively increase river flood risk in the future. At the same time, projected social and economic growth further increases exposure to flood events. Adequate flood risk reduction strategies can substantially reduce the projected increase in flood risk with global warming. The construction of river dike systems or river retaining walls is one of the adaptation strategies. Moreover, International Standard Organizations (ISO 14007) determines and communicates, the environmental costs and benefits associated with their environmental aspects, impacts, and dependencies on natural resources and ecosystem services. The standard is designed to be used in a range of applications that inform environmental management. It can be noticed that it is needed to develop a method of CBA and share a case study to facilitate understanding of the procedure because actual case studies are limited so far.

This research aims to assess the sustainability of reinforced concrete (RC) river walls in terms of environmental impacts, economic issues, and cost-benefit analysis.

To achieve the aim of this study, life cycle assessments (LCA), life cycle costs (LCC), and a GIS-based Hydrological Modeling will be implemented. This study will assess environmental impact using SimaPro software and involve a full life cycle material assessment from Cradle-to-Grave, covering raw material extraction up to end-of-life stages. The functional unit will be described as a linear running meter of the wall. Using openLCA software, LCC will be estimated as the expenditures related to material and activities along wall life spans based on their present value (PV) given as Internal cost in Japanese Yen (JPY). Furthermore, the environmental impact obtained from the LCA calculation will also be converted into the external cost of the wall. Both costs would sum up to the total cost of the wall. The benefit will be considered as Damages Avoided by evaluating physical damages under scenarios with and without the project, assisted by the GIS-based Hydrological Modeling as a flood map model. Balancing the cost and benefit of the RC floodwall will be performed conclusively.

This study will fill the research gap by concurrently conducting a sustainability assessment of the RC river wall considering environmental impact, cost, and benefit.

### **5.04.A LCA as an Effective Tool in Decision-making**

#### **5.04.A.T-01 A Comparative AB-LCA Evaluation of a Policy Instrument to Source Agricultural Biomass For Biofuels**

*Raül López i Losada<sup>1</sup> and Ralph K. Rosenbaum<sup>2</sup>, (1)Lund University, Centre for Environmental and Climate Science, Sweden, (2)Institute of Agrochemistry and Food Technology, Spain*

Introducing grass leys in crop rotations in arable land with depleted Soil Organic Carbon (SOC) levels offers the potential to increase SOC stocks and crop yields as a step towards sustainable farming systems while also providing biomass for biofuels. In consequence, Sweden introduced a payment for grass leys, which is now under scrutiny: SOC gains in the region have been

uncertain in practice, as the payment largely compensates farmers for leys that would have been there regardless, while it has failed to penetrate intensive arable land where SOC is depleted. Had the incentive achieved its goal, its overall environmental performance would still be an open question, as land use pressure from displaced foodcrops risks translating into environmental impacts elsewhere.

This example illustrates the need to study the system wide impacts of agricultural policy prospectively, in a framework that can capture the dynamics of environmental changes and structural production shifts to which they give rise. To this end, we propose an integrated economic-environmental approach coupling modularly an Agent-Based Model of farmer behavior, AgriPoliS, and Life Cycle Assessment (LCA). Recently, AgriPoliS has incorporated SOC dynamics validated with long-term field experiments and subsequent effects on yields and optimal N fertilisation levels. This allows for our approach to consider both the local multifunctionality of grasses in crop rotations and crop-displacement effects elsewhere in the environmental evaluation of policy instruments.

Our results show that the current payment of 500 SEK is too low to achieve any changes in intensively-managed farms, and it only compensates livestock enterprises for existing grass leys in fields that do not show depleted SOC levels. A 5000 SEK/ha payment specific to highly productive land capped at 25% of the area would achieve homogenous adoption of grass leys in intensive crop rotations, while compensating farmers for their loss of income from food crops and preventing simplification of the landscape mosaic. The policy intervention would yield a positive environmental outcome overall, but only if grasses are actively used for bioenergy purposes.

Our work contributes to expanding the environmental assessment of land-based biofuels at a time when their overall sustainability is highly debated. Doing so, we aim at improving scientific input for policymaking in the task of shaping sound strategies to source agricultural biomass for biofuels.

#### **5.04.A.T-02 Development of an Easy-to-implement Pesticide-related Food Product Label: Potential and Limitations**

*Cédric Furrer, Thomas Nemecek, Laura Iten and Gerard Gaillard, Agroscope, Switzerland*

There is a rising interest of customers to be informed about what they eat and how it is produced. Retailers and supermarkets have realized such needs and start to introduce labelling systems to facilitate customer information. According to customer surveys, a label, which assesses the ecotoxicological consequences of pesticide use from food production, is of great interest and has not yet been introduced. We therefore aimed to develop an easy-to-implement method, which outlines possible impacts of pesticide use from agricultural production of plant-based food products.

The ecotoxicity potential of food products was estimated using the pesticide consensus approach as defined by the OLCapest project. Characterization factors from USEtox, OLCapest and EF were adapted to consider the emissions to the different compartments (air, agricultural soil, natural soil, surface water) and linked to the active ingredients present in the lists of legally approved plant protection products (LPPP) of different countries. Ecotoxicity potentials were calculated using different types of information from LPPP. Several options were tested to aggregate ecotoxicity potentials in order to avoid overestimation.

Aggregated ecotoxicity potentials were transferred into a scoring system classified by five groups with fixed percentiles and a plausibility check was performed.

The developed "Method M-Check PPP" evaluated exclusively the freshwater ecotoxicity potential for aquatic organisms and provided plausible results for the investigated food products within the range of production- and label systems. Although a clear pattern was not observed, the different means of aggregation (i.e. median or arithmetic mean) seemed to affect scores less than different data availability. Aspects such as e.g. the use of robust crop varieties, specific breeding programs, yields, site parameters (e.g. soil properties, rainfall, etc.) could not be included due to missing data or limitations in the method.

An under- or overestimation of the ecotoxicity potential compared to reality is possible because currently only theoretical data from LPPP's is used. Aspects such as the availability of information from LPPP's of different countries, the integration of new LPPP data into the ecotoxicity calculation, and the rating of compound foods are some of the points that have not been conclusively clarified and need to be further elaborated in the future.

#### **5.04.A.T-03 From a Farm Specific Diagnosis Tool to Farm Specific Improvement Paths and a Prioritisation of Subsidies**

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Climate change is a major global challenge for agriculture today. Although it is the prime sector to be affected by climate change, it is also under rising pressure of both, policy makers and consumers, who demand a sharp decrease of the climate impact of our food, even so in Flanders. The need for more climate-friendly and climate-robust farm management is strong, especially for dairy farmers who are bound by a Covenant committing to reduce greenhouse gas (GHG) emissions and more specifically the methane emissions with 27% (compared to 2018) by 2030. Given these challenges, the sector insisted on a tool to support dairy farmers in reducing their impact on climate. But insight is not enough to result in action. Farmers run a business and they need to assess the impact of a business change -whether an investment or managerial- in terms of technical feasibility and economic viability prior to implementation. Additional support beyond diagnosis is essential. For policy makers, it is key to know what significant mitigation strategies are not cost efficient in order to better target their subsidies. An evidence based decision tool will help both parties and enable GHG reduction. To answer to these challenges, an LCA-based climate scan for dairy farms (Klimrek) was developed. The scan includes scenario analysis with cost-benefit information on proposed mitigation measures, and has proven to be an effective instrument to offer dairy farmers insight regarding the environmental impact of milk production at farm level, and support them in the selection and implementation of cost-effective climate measures. Important success factors of the approach are (1) the co-creation process -from initial scope and definition drafting a system analysis - up to interpretation of the results, (2)

a web based LCI with validation and automatic detection of possible mitigation measures, (3) cost-benefit information and additional farm specific indicators listed alongside the results, and (4) guidance from a consultant from start to finish.

#### **5.04.A.T-04 LCA Tool to Explore Emission Mitigation Options of Pig and Cattle Production at Farm Gate**

*Annika Erjavec, Jannick Schmidt and Iris Weidema, 2.-0 LCA consultants, Denmark*

Livestock production is a major driver of climate change, representing approximately 14.5% of all anthropogenic greenhouse gas (GHG) emissions with the demand for meat products predicted to rise in the future. Consequential life cycle assessment (LCA) is used in this study to develop a tool that enables the user to explore the environmental impacts of various production methods for pigs and cattle. This is a useful decision-making tool for farmers to improve production, for companies to build industry standards or governments to create policies in this arena.

Consequential LCA methodology is used in this study to calculate the environmental impacts from demanding an additional kilogram of live weight pig or cattle at farm gate. By-products such as animals to rendering and manure are modelled using substitution. The background database used is the input-output database EXIOBASE v.3.3.16b2 and the environmental impacts are calculated using Stepwise 2006 v. 1.07. The tool is Excel-based and the user can insert any combination of input data and the results will be presented immediately. The tool calculates a) country-specific national baseline emissions, b) farm-specific emissions, or c) a combination, where if the farmer does not or cannot provide every single data point, the model will populate the remaining data with national average data.

The tool contains several modules, including: a crop module that calculates field emissions at farm level related to fertiliser and crop residue inputs, with a basis in emission models from IPCC 2019; and an animal module that models emissions from housing and storage, again taking a basis in IPCC 2019 and using additional emission models where supplementation is necessary.

This tool is unique as it enables the user to alter the input data to explore different scenarios. By providing quick, visual and easily digestible results, people outside the scientific community can understand the effects of changing farming practices. On a governmental or EU level, policy-makers can explore the effects of different practices, and by gaining the knowledge of these effects, solutions such as financial incentives can be implemented. This demonstrates that consequential LCA plays a key role in creating science-based models that can quantify the effects of various farming practices and provide decision-making support on multiple levels, especially in modern times where it is vital to decrease our environmental emissions.

#### **5.04.A.T-05 Life Cycle Analysis to Determine the Trade-offs Between Food Waste Reduction and the Implementation of Monitoring Technologies**

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The importance of reducing food waste has been recognised worldwide not only because food waste causes economic severe impacts but also due to environmental and social consequences. Significant carbon emissions result from the production of food that is wasted, and the wasted food will emit more GHG in landfill, causing significant environmental impacts. Recent research supports the importance of using smart technology such as the Internet of Things (IoT) to help reduce food waste by monitoring food quality, managing food close to its shelf life, and improving the management of inventory and store layout. However, although integrating IoT technologies to monitor food quality conditions can have many advantages, the environmental implications of using these technologies have been scarcely debated. Therefore, the goal of this assessment is to understand the trade-offs between optimising the refrigeration monitoring of an abattoir in Ireland using IoT technologies to reduce food waste and its potential environmental impacts through the Life Cycle Assessment (LCA) methodology. The meat manufacturing company is located in Ireland and produces meat from three different animals' livestock, cattle, sheep and swine. The activities related to the meat manufacturing company serves as the baseline and the second scenario includes the IoT technologies used to monitor the food quality conditions in the dry ageing chambers during the cold storage process. The characterisation factors and the impact categories used in this study are those of the ReCiPe method. According to the results, the strategy to integrate IoT technologies to monitor temperature/humidity conditions did not adversely affect the organisation in a significant way. In addition, if the reduction in meat waste reaches 1%, then, the avoided environmental impacts resulting from this strategy could avoid a significant amount of carbon dioxide emissions per year, i.e. 35071 kg of CO<sub>2</sub>-eq. Therefore, this methodology proved to be suitable for determining environmental impacts and savings of food companies and can equip decision-makers to understand the environmental performance of their companies through a comprehensive and science-based methodology.

#### **5.04.B LCA as an Effective Tool in Decision-making**

##### **5.04.B.T-01 Relevance for Society and Decision-support of Attributional and Consequential Life Cycle Assessment**

*Thomas Schaubroeck, Luxembourg Institute of Science and Technology, Luxembourg*

A main goal for any sustainability assessment method is to be relevant for society and support decision-making. We aim to evaluate this specifically for two methods that assess product system sustainability and possibly support the choice of product selection in different ways: attributional and consequential life cycle approaches. We build further on evaluations from literature, but also deviate from them and expand the analysis. One of the differing aspects in our work, is that attributional and consequential life cycle assessment (LCA) are consistently considered as exactly defined by the UNEP-SETAC. Concisely, attributional LCA (ALCA) assesses the global impact share of a product life cycle and consequential LCA (CLCA) covers the consequential impact of a decision. In this conducted analysis, we first select criteria for evaluation and then, second, specify to which extent they are fulfilled by both approaches. It is quintessential to make a distinction between ideological and practical criteria. From an ideological perspective, CLCA aims to address realistic effects of the studied decision, implying complete



alignment with a first criterion on realistic modelling, desired in a sustainability assessment. Similarly, it covers a pure scientific thinking and follows fully consequential ethics on a conceptual level. Attributional LCA, does not completely align with these matters. The main reason is the rules that are behind ALCA. For example, a complete product life cycle needs to be covered, considering the past before product provision. However, following rules aligns with so called rule-based ethics, which are prevalent in society. The ALCA rules can be regarded in the same way, but society-wide acceptance is lacking. Given this potential complementarity, different ways of decision support are ideally possible. In practice, ALCA may be more readily applied because of its bigger uptake in standards and broader set of databases, but at least one database version exists purely for CLCA. Yet, it seems a misconception that the uncertainty and complexity of CLCA modelling would be way higher, since also in ALCA complex processes need to be considered as also the world harbors these and changes over time (e.g. the market mix does not stay constant). Overall, it is quite clear that both ALCA and CLCA can be of relevance. Yet, ALCA mainly needs to address acceptance of its rules from an ethical viewpoint, and CLCA needs to be more implemented in practice.

#### **5.04.B.T-02 Is data sharing in LCA FAIR?**

*Agneta Ghose, Department of Planning, Aalborg University, Denmark*

In the light of rising awareness, and growing need of better data management across disciplines, it is pertinent to investigate how the FAIR principles are utilized in the LCA domain. The purpose of this research is to investigate the status quo of data sharing by LCA practitioners. This study will look into data shared in relation to research outputs (e.g. peer reviewed articles). The life cycle inventory (LCI) is the most common data that is re-used, hence this study investigates how the LCI is shared in peer reviewed articles. A review of the findability, accessibility, interoperability and re-usability of the LCI data of 25 peer reviewed articles was performed. This study highlights although there is growing awareness on the importance of data sharing, the lack of a clear guidelines within the LCA domain as well as limited infrastructure is a major barrier in implementing FAIR principles. Research data sharing is beyond the sole responsibility of individual researchers. The overall research and funding infrastructure must consider the diversity of practices, differences in fields, facilitate the sharing of a broad range of research outputs. Current gaps in academic data sharing can be seen as opportunities to build a common framework.

#### **5.04.B.T-03 Life Cycle Assessment, Quo Vadis? Is There One Right Way of Performing LCA? Preliminary Results from a Survey**

*Miguel Brandão, Royal Institute of Technology, Sweden*

Life cycle assessment (LCA) has been recognised as an important environmental systems analysis tool due to its potential for providing holistic results about the environmental impacts of alternative production and consumption systems that can lead to decisions towards greater sustainability in both private and public-policy contexts. However, LCA has been under increased scrutiny due to the wide range of published results on similar systems, such as biofuels, which can be contrasting. This variability is, in part, due to the proliferation of guidelines that have emerged over the last 20 years, and it has been suggested to undermine the robustness of LCA as a decision-support tool. Following some interesting discussions on this topic in different fora, we took the pulse of the LCA community on this topic via a survey. We received around 100 responses from respondents who varied in their background and experience in LCA (most were academics and/or had more than 10 years' experience), as well as in their opinions on whether they saw the inconsistency of published results problematic or not for decision making. Preliminary results suggest that respondents are of the opinion that i) there is no one right way of performing LCA; ii) the ISO 14040-44 standards were insufficient for guiding LCA practice; iii) the large flexibility with which practitioners perform LCA undermines its validity as a robust tool for decision making, and that iv) further efforts in harmonizing LCA practice would be beneficial, despite the mixed opinions shown by respondents, which indicates the divisive nature of this topic in the LCA community. Further harmonization would help to ensure consistency in the application of the tool by practitioners which, in turn, would ensure results would be less variable and arguably more meaningful. It is likely that the associated methodological issues remain unresolved in the near future.

#### **5.04.B.T-04 Using LCA as a Tool for Investigating and Enhancing the Net Negative Emission Potential of Climate Positive Technologies**

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Climate-positive technologies (or Negative emission technologies) are technologies that remove carbon dioxide (CO<sub>2</sub>) or methane (CH<sub>4</sub>) from the atmosphere thus generating so-called negative emissions. These have been highlighted as a key component of nearly all pathways to limit warming to 1.5°C above pre-industrial levels. However, a challenge in this area is the evaluations of both techno-economic and environmental performances which are key to understand opportunity and unravel challenges. Amongst these aspects, there is a need to evaluate their environmental performances to determine the net negative emission potential considering impacts from their whole specific value chains using a consistent system boundary. The need for a consistent system boundary to include all upstream and downstream emissions is essential to evaluate the actual negative emission potential and to avoid underestimation.

The present work aims at developing a framework for a fair and transparent benchmarking of climate-positive concepts to identify the most promising technologies based on a standard set of guidelines. Many of these climate-positive technologies are currently in a conceptual phase and we propose LCA as a tool for optimizing the value chain in order to minimize negative environmental impacts, as well as avoid problem shifting.

By developing conceptual LCA models of selected climate-positive pathways, the environmental hotspots of these emerging technologies will be identified. This in turn will facilitate decision making regarding choice of processes, source of energy,

location, scale, materials and end of life treatment. The wider impacts of some these pathways on other systems like food value chains and energy production will also be investigated to give a complete picture of the indirect impacts. This work will further look into which type of LCAs (attributional, consequential, comparative) are suitable for the decision-making process based on the user group i.e. technology developers, project owners, policy makers, regulatory bodies etc.

Benchmarking the CCS technologies while understanding the associated positive and negative consequences will provide a solid knowledge base for developing future national GHG reduction strategies. Furthermore, a detailed environmental LCA will help foresee if the technologies have any negative consequences on other impact categories than global warming potential and help recognize the problem of burden shifting early on.

#### **5.04.P-Tu381 Modelling of Electricity in Product Environmental Footprints**

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The EU framework for Product Environmental Footprint (PEF) is now being referred to in EU legislation. Requirements on the maximum carbon footprint are included both in the draft Ecodesign Regulation for photovoltaic (PV) modules and inverters, and in the Commission proposal for a new Battery Regulation. The climate impact of these products will be calculated using the PEF methodology. Since this will be the first time a piece of legislation using the PEF methodology comes into to force, it may become a precedent for future use of PEF in a policy context. However, the rules for modelling the electricity supply in the draft PV Regulation do not reflect the calculation rules in the most recent PEF guidelines. These rules, in turn, have been criticized for lack of clarity and for allowing for greenwashing. We investigate the need for revised texts regarding the modelling of electricity production in the EU Ecodesign Regulation for photovoltaics and in the general PEF rules. We also produce suggestions for such revisions. These revisions will also have an impact on the battery regulation since this also refers to the PEF method. Through interviews with various actors, we gather information on what is unclear and controversial in these texts and suggestions on how to improve them. In a literature study, we compare the draft PV Regulation to various versions of the general PEF guidelines and to the Product Category Rules (PEFCR) for PV. This serves the purpose to better understand the origin of the text in the draft PV Regulation and to investigate how this text should be revised to reflect the current PEF methodology. To identify alternative rules and explanations of these rules, we compile information on how the electricity supply is modelled in various other frameworks: Environmental Product Declarations, the EU Renewable Energy Directive, etc. Early results indicate that deviations in the draft PV Regulation from the general PEF rules are in part deliberate abbreviations of the text. However, an up-to-date Regulation should allow for the use of data representing the residual mix in the EU or the region where the electricity is used. It should perhaps also not require the use of national data from the Sphera database. The general PEF rules should be supplemented with additional guidance for LCA practitioners on how to interpret and apply concepts such as contractual instruments, tracking, and tracking systems.

#### **5.04.P-Tu382 Determining Missing Life Cycle Inventory Data Using the Research – Reaction – Energy – Modeling (RREM) Approach: An Example of Surfactants in Cosmetics**

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In life cycle assessment (LCA) the granularity level of the underlying inventory data can greatly affect the results. Specifically, this refers to the modeling of products made from complex chemical compounds, such as personal care products and cosmetics. While existing LCA databases contain life cycle inventory (LCI) data for most common organic chemicals, more complex substances are often represented only at the level of chemical classes or in accordance with their function (e.g., non-ionic surfactants).

Existing approaches for modeling missing inventory data are either based on molecular structure and thus do not comply with a life cycle perspective, or are only applicable to compounds with relatively small shares of a product, given their high uncertainty level. The previously published Research – Reaction – Energy – Modeling (RREM) approach provides a novel framework for establishing process-based LCI for chemicals in the absence of primary data.

In this work, an updated version of RREM will be tested on an example of common surfactants in cosmetics derived from fatty acids divided into sulfonates, glucosides, and glycols. Thereby, the global warming potential (GWP) of the modeled substances will be compared with that of corresponding generic datasets available in LCI databases as well as RREM-based “special proxies” (s-proxies). Additionally, a sensitivity analysis will be carried out to evaluate the impact of assumptions.

Preliminary results indicate an underestimation of GWP results by 2 to 4 times when applying generic datasets to model fatty acid sulfonates. In contrast, the results for glucosides modeled by RREM are nearly twice as low compared to those of generic datasets. For glucoside-based surfactants, no clear trend has been observed. In light of the previously published results, it is likely that RREM-based proxies overestimate the impact assessment results.

RREM approach may be a promising way of modeling the environmental impacts of complex chemicals from a life cycle perspective with relatively little effort. RREM as such is not directly linked to the FAIR-principles, but could be adapted to them. The next step should involve obtaining statistically significant results for evaluation of the robustness of the approach. Further, a more sophisticated set of assumptions for building s-proxies should be provided, such as those pertaining to reaction types.

#### **5.04.C LCA as an Effective Tool in Decision-making**

#### **5.04.C.T-01 Review of Life Cycle Assessments for Maritime Fuels**

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As part of an increasing awareness of the detrimental environmental effects of the use of fossil fuels for maritime transport, the sector's emissions have been met by targets on regulatory and company levels, with the International Maritime Organisation (IMO) setting reduction targets of 50% of these emissions by 2050 when compared to 2008.

Alternative non-fossil based fuels have been identified as a key lever through which to reduce the maritime transportation sector's carbon footprint. These fuels include biofuels and electrofuels, like hydrogen and methanol. The environmental performance of these fuels – in areas other than just climate change – should be a deciding factor in their adoption in the sector's green transition. Life cycle assessment (LCA) is an effective methodology tool for assessing this environmental performance and have been used in decision-making in various sectors. Several LCAs of maritime fuels have been conducted. However, assumptions made when conducting these LCAs can have substantial effects on the results, and therefore need to be well documented and transparent. With the aim of providing insight into the state of the existing research on the environmental impacts of maritime fuels, a systematic review of existing maritime fuel LCAs published in peer-reviewed journals was conducted.

The review conducted revealed key gaps to be addressed in future LCAs of maritime fuels, including a lack of transparency when reporting key elements of the LCAs, such as an underreporting of the functional unit. Most of the LCAs assessed in the review only considered climate change, resulting in little information about other potential environmental impacts. For example, uncertainty analyses were not conducted in most of the reviewed LCAs, which is important to ensure the robustness of an LCA study.

Following the gaps identified in the review, an LCA modelling framework will be created to ensure inclusion of all important factors and limit the potential of burden shifting of environmental impacts. The framework aims to be comprehensive and systematic, and provide a guideline for decision-making with regard to fuel for maritime transport.

#### **5.04.C.T-02 LCA-based EU Policies For Low-carbon Hydrogen Deployment: Risks And Opportunities**

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Future EU policies aimed at reducing greenhouse gas emissions will likely rely on life cycle assessments to determine the carbon footprint of products and activities. This can lead to significant benefits in terms of carbon accounting, which is crucial for reaching planned climate targets, avoiding carbon leakage, and greenwashing. On the other hand, the different approaches currently available for LCA-specific methodological choices (e.g., how to handle multi-functional activities in the ISO standards) can have unwanted consequences; specifically a broad range of carbon intensity for hydrogen and its co-products. In this paper, we showed how hydrogen co-produced with methanol could have a carbon intensity varying from 1 to 11 kg CO<sub>2</sub>e/kg H<sub>2</sub>. This could create distortion in the market and hinder optimal solutions in terms of climate policies. With this contribution we aim to raise awareness on the necessity of strict and harmonized life-cycle based guidelines for each activity covered by the EU climate policies. At the same time, we want to engage the LCA community in supporting the European Commission in the choice of a LCA approach leading to a minimization of the climate impacts.

#### **5.04.C.T-03 Ecodesign Approach Based on Prospective Life-cycle Assessment, To Support R&D Decision Making For New Generations of Batteries**

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The highest levels of environmental performance are achieved when environmental criterion is introduced in the early stages of the design and development process of new technologies. Thus, the availability of environmental information throughout their lifecycle is fundamental for ecodesign and the LCA methodology can provide this information. However, during design decision making in a R&D context, LCA is mainly applied at a low level of technology maturity and for researchers this remains a difficult requirement to consider. This work aims at responding to the challenge of environmental performance criteria integration into technological developments of future generations of batteries and will be applied to three case studies: the advanced Li-ion, Lithium-Sulphur and all-solid-state battery technologies. The objective is to propose a methodology to support researchers through ecodesign decision-making by helping them to understand the environmental issues and to identify the levers for improving the environmental performance of new generations of batteries.

The Design Research Methodology (DRM) framework proposed by Blessing and Chakrabarti (2009) was adopted. The DRM application leads on, among others, the definition of resources and needs in terms of environmental assessment as well as action levers and limits in ecodesign for this R&D context. This results in the identification of three moments of support where environmental evaluation is needed: project set-up, daily R&D tasks and final evaluation of the technology. To meet this needs, the proposal is based on a ecodesign toolbox built on adapted R&D prospective LCA principles which consists of four operational tools for the research teams: A Li-ion batteries environmental impacts database for the 3 new battery technologies and commercial reference technologies. A simplified and parameterised assessment tool where modelling can be performed depending on the researcher context and with a specific assessment of material criticality. An ecodesign suggestion bank which presents a collection of ecodesign tools. And a series of short videos for researcher awareness and training about the issues and challenges arising from the battery sector.

The set of tools that support the new method are being developed and their usability will be validated during the last phase of the DRM. It is expected that this proposal will guide researchers in their decision making all along the design process.

#### **5.04.P-Tu383 LCA-based decision support on the inclusion of mechanical vapor compression in early phases of process design**

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Worldwide, chemical industry has set a pathway to the conversion to a CO<sub>2</sub> neutral production. The electrification of processes to substitute fossil-based energy supply may contribute significantly to achieve this goal. This needs the development and implementation of new technologies and the transformation of traditional fossil heated processes. One of those technologies is the Mechanical Vapor Recompression (MVR), which can be used to upgrade waste heat for heat integration in thermal separation processes and thus reduce the externally heat demand substantially.

The complexity of existing installations and the missing experience with those new technologies regarding process design and implementation in thermal separation processes hamper a fast and comprehensive integration of these new technology. Other obstacles, like the lack of good working examples or high investment, hinder a wide application of this technology yet. To assess the sustainability of an MVR implementation into the development of a new or the revamp of an existing process, expertise from different fields needs to be combined.

This contribution presents the development of a comprehensive method, which shall support process engineers to decide at an early design stage, whether and how the implementation of MVR in existing or new rectification columns proves beneficial from a technological, environmental and economic perspective. By combining the expertise of process engineers, compressor experts, and LCA practitioners different process concepts and equipment installations are compared in terms of costs and environmental impact. The approach comprises of an exergy analysis of the given separation problem, the design of optimal compressors and heat exchangers, economic and environmental assessment of different equipment options and a visualization of the results. In further work a tool with a graphical user interface is planned with several default values and assumption of state-of-the-art process parameters, intending a direct and low-threshold usage of the method.

The method offers a decision support for engineers and managers working on optimization and transformation of fossil-based processes to electrification by an early-stage quantitative assessment of the potential of MVR in distillation columns.

#### **5.04.P-Tu384 LCA as a Guide and Decision Support in Technology Research and Development Projects – The Case of the ELSAH Project**

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A multitude of European research projects aim at the collaborative development of novel and innovative technologies and products. LCA is being applied as one of the primary tools to demonstrate and better understand the potential environmental performance of outcomes, but also to guide the development process itself, from an evidence-based environmental perspective, as it progresses towards higher technological readiness levels. A flexible approach concerning the assessment methods and scope allows support that may range from initial material screening to full LCA implementation including all life cycle stages.

This presentation illustrates the ELSAH EU project as a case study, where LCA is being used to guide the development of a wearable sensor system (for monitoring of molecular biomarker concentrations) towards more environmentally friendly solutions. Cradle-to-gate assessments were applied to subsequent generations of individual components, and integrated or semi-integrated demonstrators, to identify impact hotspots and evolution. A full LCA will be performed of the final prototype towards the end of the ongoing project. However, as is often the case, small-scale and non-industrial production methods associated with research projects, and the lack of real data concerning product use and end-of-life stages, severely limit a more holistic and conclusive impact overview in a broader context and relative to potential competing solutions. Consequently, it is argued that more traditional LCA approaches may be useful to inform project-internal decision making towards more sustainable outcomes, but to facilitate effective broader perspective decision making there is need for more advanced methods, such as theoretical foreground process upscaling and background future scenario modelling, as promoted in prospective LCA approaches.

#### **5.04.P-Tu394 The Agrifood Data Sharing Platform as Pivot for LCI and LCIA Results**

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An LCA-based climate scan for dairy farms (Klimrek) has proven to be an effective instrument to offer Flemish farmers insight regarding the environmental impact of milk production at farm level, and support them in the selection and implementation of cost-effective climate measures. The large need for data associated with the LCA approach, however, makes completing the scan time-consuming, which hampers its roll out on a larger scale. Initial experiences with the scan, which is completed by consultants during a farm visit, show that manually entered data is subject to errors and interpretation. This can affect the correctness and fairness of the scan's results. Automatic input of existing data from other digital data sources, using the Flemish data sharing platform DjustConnect, offers a solution for these problems. DjustConnect is an API-based data sharing infrastructure, that allows exchange of data between data users and providers, only with consent of the farmer. With the climate scan as a catalyser, different data providers made their data available via APIs on DjustConnect. With consent of the farmer, their data can be used to complete part of the life cycle inventory (LCI). The farmer can also use the data sharing platform to share the scan's results and (in the future) export part of the collected LCI data to other digital tools. The LCI is designed to make maximum use of standardised terminology, in order to make data available for other applications. To take full advantage of the benefits of data sharing platforms, consistent use of data standards and farmer acceptance remain important challenges that need to be addressed. At the

moment, few data sources use standardised terminology, hampering the easy exchange of data between tools. Besides this, many Flemish farmers are still hesitant to trust and use data sharing platforms. Information campaigns and success cases like the climate scan can boost this lack of confidence and raise awareness for the benefits of safe data sharing.

#### 5.04.P LCA as an Effective Tool in Decision-making

##### 5.04.P-Tu385 A Life Cycle Assessment of a Remanufactured Bicycle at An Mheithal Rothar, a Community Cooperative Bike Shop

*Cathy Coote<sup>1</sup>, Cherrelle Johnson<sup>2</sup> and Sinead Mitchell<sup>3</sup>, (1)An Mheithal Rothar, Ireland, (2)Mechanical Engineering, National University of Ireland, Galway, Ireland, (3)Management, University of Galway, Ireland*

In Ireland, cycling is growing steadily in popularity and the Program for Government plans a €360 million spend on the development of active travel infrastructure, which will lead to increased demand for bicycles. However, recent supply chain disruptions are making it harder to import bikes, and to export waste. A remanufactured bike can save metals and mixed materials from entering the recycling or landfill waste streams.

Remanufacturing can prevent impacts related to the extraction, processing and transportation of virgin material. Remanufacturing is not simply repairing or refurbishing, but through a factory process, as good as new (or better), with a warranty, and you cannot tell the difference. The production stage of a bike has the highest negative impact. A remanufactured bicycle has the potential to generate better environmental benefits than recycling and greater economic output than the materials alone. The markets to export waste materials are closing and may become unavailable to Ireland.

A simplified comparative Life cycle assessment (LCA) was performed using the Environmental Footprint Methodology (EF 3.0) and EcoInvent database v3.9. This LCA was based on a remanufacturing process that restored the highest value parts of a recovered bike from municipal waste, namely the frame, fork and handlebars. The remaining parts were replaced with new ones (e.g. brakes, wires, bike chain) to guarantee the remanufactured, like new or better product output. The bicycle production process data set represents a bike of 17kg including common additions.

The LCA compared a newly manufactured bike to a remanufactured bike, which demonstrates approx. 50% reduction in emissions for the “as new” bike by An Mheithal Rothar, estimated total Equivalent CO<sub>2</sub> emission savings of 201.04 Kg CO<sub>2</sub>-eq. This result will support the decision to scale up production of remanufactured bikes, and guide future development of circular economy solutions and inform circular economy enterprises, policymakers and government of the benefits of remanufacturing to achieve decarbonisation, emissions reductions and increased circularity rates in Ireland.

##### 5.04.P-Tu386 Applying Circular Economy Principles to the Problem of International Catering Waste – A Life Cycle Assessment Case Study of the Aviation Waste Sector

*Lois Pennington<sup>1</sup>, Andrew Welfle<sup>1</sup>, Ben Parkes<sup>2</sup> and Nigel Scrutton<sup>3</sup>, (1)Tyndall Centre for Climate Change Research, University of Manchester, United Kingdom, (2)Department of Mechanical, Aerospace, Civil and Environmental Engineering, University of Manchester, United Kingdom, (3)University of Manchester, Manchester Institute of Biotechnology, United Kingdom*

The advent of commercialised flights has brought an increasing demand for international travel. Alongside the effect of increased fuel burn on the climate, the growth of air travel has brought an additional problem; the management of cabin waste. Many countries have strict legislation controlling the disposal of this waste, due to the possible presence of animal by-products such as meat or dairy derivatives. The risk of cross-contamination onboard means all cabin waste from international flights is classified as high-risk (CAT1) International Catering Waste (ICW). Previous studies estimate that up to 80% of cabin waste is either recyclable or compostable. As a result, in areas such as the EU and the UK, it is currently only permitted to either send this waste to landfill or incineration. The volume of waste generated and the resultant environmental impact of its disposal is currently not required to be reported. There is also no evidence of a risk or impact assessment being undertaken before the implementation of this legislation.

This research, developed in collaboration with Manchester Airport, aims to quantify the volume of cabin waste entering the UK and the impact of existing legislation on its disposal footprint. Initial estimates show that around 116,000 tonnes of waste are generated yearly on incoming flights to the UK; a similar volume as would be generated by a city such as Belfast. Life Cycle Assessment methodologies are applied to existing disposal methods to estimate the environmental impact of this cabin waste. These results are compared to speculative disposal pathways if the policy were to be changed to allow the application of circular economy principles to this waste; prioritising reuse and recycling, and the generation of bioenergy through anaerobic digestion. In total, eight scenarios are modelled for comparison. The LCA is completed using SimaPro software and a combination of data from literature, the EcoInvent 3.7 database and pilot plant results.

The results emphasise the highly negative environmental impact of landfill & incineration as disposal methods. It quantifies the reduction in Greenhouse Gas emissions that could be achieved by the implementation of more risk-based measures and Circular Economy Principles. The use of LCA in this project aims to provide a data-based evidence source to inform policymakers, responding to recent calls by airlines and the International Air Transport Association for smarter regulation of cabin waste.

##### 5.04.P-Tu387 A Novel ‘Hub and Spoke’ Framework for the Holistic Sustainability Assessment of Chemical Value Chains

*Alex James Kenneth Newman<sup>1</sup> and Peter Styring<sup>2</sup>, (1)University of Sheffield, United Kingdom, (2)Chemical and Biological Engineering, University of Sheffield, United Kingdom*

Sustainability assessment has emerged as a critical sub-field within ongoing industrial ecosystem development. This has led to the question; ‘How can explorative and holistic value chain-oriented sustainability assessments support strategic decision making?’. Guidelines targeting isolated life cycle analyses (LCAs) and techno-economic analyses (TEA)s currently dominate, neglecting

both their integration and the addition of social impact analysis (SIA). This shortcoming is manifested through inter-strand burden shifting, negating any improvements in the assessed strand. An ISO 14K aligned framework is needed for holistic, modular assessments of complex value chains.

The developed methodology builds upon GCI's "Techno-Economic Assessment & Life Cycle Assessment Guidelines for CO<sub>2</sub> Utilisation" and the McCord et al. "Triple Helix Framework". Assessments comprise of interchangeable modules; core processes, or 'hubs', and input/output flows, termed 'spokes'. In this, a 'plug and play' approach to industrial value chain modelling is developed. Thus, competing value chains are fairly and robustly compared with congruent system boundaries and assumptions. Inventory sheets are completed for each of the spokes considered, and models of the hub processes constructed. Multiple production or disposal routes may be assessed for each flow crossing the hub's boundary; these are grouped into 'spoke sets'. Life cycle impact analysis (LCIA) is completed for each spoke in isolation, generating indicator results relative to a local functional unit. These indicator results are then scaled to the relevant hub's reference flow. When aggregated, these modules generate assessments of the full value chains.

To facilitate tailored value chain recommendations, spokes' indicator scores are normalised within the set in which they are a constituent. Multi-criteria decision analysis (MCDA) in the form of tiered analytical hierarchy process (AHP) is employed to identify the best performing spokes in the context of decision maker value choices. Assessment results are generated in two parts; traditional objective results (impact indicators), calculated via characterisation models, and a subjective MCDA derived overall score, aggregating normalised indicator scores using AHP weightings. To conclude, the methodology will provide industry with a decision-making tool aimed at the strategic development of incoming sustainable value chains while maintaining granularity and robust objective results.

#### **5.04.P-Tu388 Sustainable Comfort on Board – Using LCA Data for a New Cruise Ship Cabin Design**

**Mareike Tippe<sup>1</sup>, Urte Brand<sup>2</sup> and Thomas Vogt<sup>2</sup>, (1)DLR-Institute of Networked Energy Systems, Germany, (2)Energy Systems Analysis, German Aerospace Center, Institute of Networked Energy Systems, Germany**

According to an agreement of the International Maritime Organization (IMO) the shipping industry is supposed to cut its CO<sub>2</sub> emissions by not less than 50% by 2050. With shipping contributing to a great degree to climate change, diverse measures have to be taken in order to decrease these emissions. Following a different approach than solely focusing on the propulsion system of ships, in the project EcoCab (funded by the German Federal Ministry for Economic Affairs and Climate Action) decentralized cabin-air and energy-supply concepts were developed together with the ship-building yard MEYER WERFT GmbH & Co. KG. With up to 3500 cabins per cruise ship, their usage and material composition significantly contribute to the environmental footprint of the ship.

Since the cabins serve a strong representative purpose for the shipping company while also being the passengers' private space on board, major changes of those utilities go along with a diverse set of challenges that relate to all dimensions of sustainability. Not only focusing on the decentralized energy supply and air conditioning, but also on the use of cradle-to-cradle materials for the cabin structure and interior, a multidimensional assessment framework was set up to compile data on environmental, economic, social, technical and legal matters. LCA data were combined with assessment results from the other dimensions in form of a multi-criteria decision analysis (MCDA).

Results showed, that design and construction options with the – according to the LCA – least environmental impact often interfere with either legal requirements (e.g. IMO guidelines and norms) or matters of acceptance when it comes to the installation of e.g. photovoltaic modules on the cabin balcony. Also, standardisation of construction and building processes impose a great challenge, that has to be considered when making decisions on the implementation of environmentally friendly options.

Our contribution to the conference will focus on the methodological approach of conducting an LCA and processing the according data within a multidimensional assessment framework and MCDA. Next to the presentation of LCA results of a complex, multi-component system that the ship cabin constitutes, insights and experiences from the result integration in the decision-making and communication process will be shared.

#### **5.04.P-Tu389 NEPTUNUS-WEF1.0: A Friendly Tool in the Decision-making Process Related to the Seafood Production and Consumption**

**Sandra Ceballos-Santos, Jara Laso, Laura Ulloa, Maria Margallo and Rubén Aldaco, Department of Chemical and Biomolecular Engineering, University of Cantabria, Spain**

In the current and future context of global competition for limited resources to meet the demand for freshwater, energy and food, the habits and actions of individuals, companies and governments must be rethought and adapted. Consequently, most industries, such as the European seafood sector, will require a modernisation of the current trajectory to gain resilience and cope with global competition, undervalued prices, fishing quotas, but also adapting fisheries to climate change. Against this backdrop, stakeholders in production sectors are increasingly turning to environmental certification standards, also called eco-labels, to stand out and to reach consumers, in the belief that these labels bring more added value to their products. In this regard, life cycle assessment (LCA) has become a flourishing methodology to measure the environmental impact of products and services. However, the long-standing strength of LCA studies can also be interpreted as a major limitation in communicating the environmental profile of a product beyond the scientific community.

To overcome this situation, the NEPTUNUS project has developed a methodology to perform environmental footprints studies of seafood products in a harmonized and consistent manner, under a life cycle perspective. The project aims to pursue a new transnational clustering concept approach to review, examine and harness key eco-labelling and key enabling eco-innovations. NEPTUNUS includes the introduction of the Water-Energy-Food (WEF) NEXUS variable in the decision-making process related to the circular economy of seafood, in addition to the typical economic, environmental, and social variables. The term NEXUS

implies that an action in one of the systems has also consequences in the others and it is therefore important to understand synergies and trade-offs to develop response options that ensure a more sustainable environment. Such methodology has been implemented in a friendly tool for producers and consumers. This simplified widget will provide the WEF NEXUS score of a wide variety of fisheries, aquaculture and processing activities through an ecolabel. The design contains an easy-to-read image composed of four footprint symbols, plus the nexus score as a percentage with a colour scale.

Using this tool both producers and consumers will be able to improve their decisions based on a science-based ecolabel that creates trust and security by enabling green and conscious choices when buying sea products.

#### **5.04.P-Tu390 Life Cycle Assessment of Fisheries: The Need for Adopting a “Fishnet Approach”**

**Gregoire Gaillet<sup>1</sup>, Aurore Wermeille<sup>1</sup>, Arnaud Helias<sup>2</sup> and Anne Asselin<sup>1</sup>, (1)Sayari, France, (2)ITAP, Univ Montpellier, INRAE, Institut Agro, France**

While Life Cycle Assessment methods are increasingly tackling impact of human activities on biodiversity, the main threat on marine ecosystems identified by the IPBES: direct exploitation of biotic resources, is still hardly included. A new method of assessing these impacts in the Area of Protection – Ecosystem Quality has recently been developed by Hélias, Stanford-Clarke and Bach. This work proposes characterization factors (CFs) linked to the exploitation of a given species in each FAO Major Fishing Area.

We propose to expand the assessment to all species caught in the fishing activity (the use of a given gear to target a given species in a given area). The goal of this paper is to i) describe how to apply a “fishnet approach” of fishing activities in the context of LCA, ii) apply it to a LCIA case study of resource depletion potential with data from a Marine Stewardship Council report on a German saithe fishery (*Pollachius virens*) and iii) discuss results, advantages, and limitations of such an approach.

Results show that the fishnet approach gives an impact per mass unit of up to  $2.95 \times 10^{-9}$  PDF.year/kg of saithe (live weight) whereas a single-species approach gives an impact per mass unit of  $2.52 \times 10^{-9}$  PDF.year/kg of saithe (live weight) for this case study. It thus better reflects the impact the activity can have on the ecosystem by combining the depletion of both the targeted stock and bycatch. Vulnerable stocks (in this case: pollack, *Pollachius pollachius*, and Atlantic cod, *Gadus morhua*) contribute greatly to the total impact under the fishnet approach despite representing a small fraction of the total catches.

The method is greatly data-demanding but estimations of potential bycatch in a given fishing métier may be found in a variety of sources (Regional Fisheries Management Organisations, FAO, National Fisheries Science Organisation). By combining the impacts on all potentially caught stocks, vulnerable and not commercialized species are assessed even if they represent a small fraction of the total catches. The use of this approach would provide decision-makers with more accurate assessments for an ecosystem-based management of fisheries.

#### **5.04.P-Tu391 Assessing the Environmental Impact of the Application of a Smart Farming System via Life Cycle Analysis – Recommending Best Management Practices**

**Thanasis Karagkounis<sup>1</sup>, Evangelia Fragkou<sup>2</sup>, George Tsegas<sup>2</sup>, Fotios Barmpas<sup>2</sup> and Nicolas Moussiopoulos<sup>2</sup>, (1)Department of Mechanical Engineering, Sustainability Engineering Laboratory, Aristotle University of Thessaloniki, Greece, (2)Sustainability Engineering Laboratory, Mechanical Engineering, Aristotle University of Thessaloniki, Greece**

The Life Cycle Analysis (LCA) presented in this study is undertaken in the LIFE GAIA SENSE project and aims to assess the potential environmental benefits of Smart Farming (SF) by producing quantified results, as well as to recommend SF-based best agricultural management practices, in the context of supporting decision making. SF is a state-of-the-art digital-based technology for agricultural management, which practically focuses on minimizing field-inputs (fertilizers, pesticides and irrigation) and subsequently, reducing the related environmental impacts. In the LCA study, comparison of the environmental performance of conventional field management practices (reference fields) with those using SF technology (treatment fields), under similar soil and climatic characteristics, for the same cultivation years, was conducted. A cradle-to-field gate approach was followed, while the inventory was based on the data monitored in crop logbooks and questionnaires handed out to smallholder farmers in Greece, participating in the project. The data included details about all field cultivation and management operations that took place in the 2020 and 2021 cultivation years. The findings indicate that, except from the cases that showed no differences by applying the SF technology, there were mainly reductions in most of the impacts studied, potentially resulting from the implementation of the agricultural advices that the SF system suggested. In terms of recommending management practices, single scores were calculated by normalizing and weighting the results with the provided set from ReCiPe 2016 (H) method. The results highlight resources (fossil and mineral) depletion as the most important impact, which is mainly decreased for the treatment fields in comparison with the reference ones, implying that SF can also lead to significant resource efficiency benefits. Recommending agricultural management practices focused mainly on fertilizers application and irrigation practices. As for fertilizers application, recommendations pointed at different fertilizer types, resulting to variations in NH<sub>3</sub> air emissions, their effect on air quality and its associated impacts, while for irrigation, at the environmental performance of different irrigation methods. Finally, the main contributors of each impact were also analyzed, in the context of examining the space, time and crop-specific representativity of the results to facilitate decision making.

#### **5.04.P-Tu392 Environmental Evaluation of a Rotation System Developed in the Two Core Farming Regions of Egypt**

**Sara Lago Oliveira<sup>1</sup>, Maria Teresa Moreira<sup>2</sup> and Sara González-García<sup>3</sup>, (1)University of Santiago de Compostela, Spain, (2)Universidade de Santiago de Compostela, Spain, (3)Chemical Engineering, University of Santiago de Compostela, Spain**

Food security is at risk worldwide. While agricultural activities are essential to ensure food availability, they can also pose a risk when practised in an unsustainable manner. Additionally, Egypt is marked by its limited availability of arable land and water stress problems. To confront food shortages in the long term, the Egyptian Government has developed the Sustainable

Agricultural Development Strategy intending to improve agricultural productivity by 2030 in a sustainable manner. In this context, this study evaluates the environmental performance of a rotational system cultivated in two different key farming areas of Egypt (along the Nile River and within the desert). This evaluation aims to understand the environmental burdens of Egyptian agriculture and specificities among regions, and based on the acquired insights, to offer improvements that could guide government decisions and policies towards more sustainable agriculture. The rotation system analysed consisted of white clover, maize, and wheat arranged in a 2-year cycle. The life cycle assessment (LCA) methodology was used from a cradle to farm-gate approach. Global warming (GW), marine eutrophication (ME), terrestrial ecotoxicity (TET), water scarcity (WS) and global Potential Species Loss (PSL<sub>glo</sub>) were the assessed impact categories, based on one hectare (functional unit). To calculate the environmental impacts, ReCiPe Midpoint v1.07 (H) method, as well as AWARE (in the case of WS) and the countryside species-area relationship (SAR) model (in the case of PSL<sub>glo</sub>) were used with the assistance of the software SimaPro 9.1. The results reveal that the rotation grown in old lands presents the best environmental profile for all categories except PSL<sub>glo</sub>, with a reduction ranging from 11% to 68%. The overall better performance of old lands is a consequence of the lower fertilisation rates applied, along with the lower water consumption. Regarding PSL<sub>glo</sub>, the significant impact in old lands (97% against 3% in new lands) is related to the substantial land use pressures on this area due to anthropogenic activities, in addition to the longer regeneration time required. Moreover, irrigation stands out as the main factor contributing to environmental burdens in new lands (e.g., 62% of GW and 100% of WS), whereas in old lands, on-field emissions from nitrogen-based fertilisers are the main responsible of overall environmental profile (e.g., 90% of ME and 53% of GW).

#### **5.04.P-Tu393 Peat Bog Recultivation and Rehabilitation Strategies in Latvian Conditions: A Carbon Footprint Analysis Implementing LCA**

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Peat bogs in Latvia account for approximately 10% of the total land area. In 2021, Latvia was the main exporting country in Europe for peat products with 2235.5 thousand tons of peat exported with a profit of 239 million euros. Although the peat industry contributes significantly to the national economy, draining bogs releases a large amount of greenhouse gas (GHG) emissions into the atmosphere, promoting land degradation, which can lead to land erosion. At the same time, peat bogs have a crucial role in carbon sequestration; globally, peat bogs store up to one-third of the world's terrestrial carbon pool. In fact, the European Union is moving towards banning peat extraction in its member states, though there is still no common methodology for estimating the CO<sub>2</sub> emissions and carbon losses created during peat extraction. National GHG emissions reports published on the United Nations website have differences in calculating GHG emissions and determination of emission factors. This can affect the fair implementation of recultivation and rehabilitation of peat fields after closing peat extraction sites. Therefore, this study aims at assessing the reduction of GHG emissions facilitated by different recultivation and rehabilitation strategies for existing peat sites in Latvia according to IPCC methodology within the frame of the life cycle analysis perspective. The study results will help promote optimal management of peatlands during peat development, develop strategies to help understand how to manage peatlands after their closure, and give suggestions towards a common framework for estimating the GHG emissions of peat bogs according to a life cycle thinking-based approach.

#### **5.04.P-Tu394 The Agrifood Data Sharing Platform as Pivot for LCI and LCIA Results**

*Anne-Sophie Sacré<sup>1</sup>, Veerle Van linden<sup>2</sup>, Reindert Heuts<sup>2</sup> and Eva Maes<sup>2</sup>, (1)ILVO, Belgium, (2)Flanders Research Institute for Agriculture, Fisheries and Food, Belgium*

An LCA-based climate scan for dairy farms (Klimrek) has proven to be an effective instrument to offer Flemish farmers insight regarding the environmental impact of milk production at farm level, and support them in the selection and implementation of cost-effective climate measures. The large need for data associated with the LCA approach, however, makes completing the scan time-consuming, which hampers its roll out on a larger scale. Initial experiences with the scan, which is completed by consultants during a farm visit, show that manually entered data is subject to errors and interpretation. This can affect the correctness and fairness of the scan's results. Automatic input of existing data from other digital data sources, using the Flemish data sharing platform DjustConnect, offers a solution for these problems. DjustConnect is an API-based data sharing infrastructure, that allows exchange of data between data users and providers, only with consent of the farmer. With the climate scan as a catalyser, different data providers made their data available via APIs on DjustConnect. With consent of the farmer, their data can be used to complete part of the life cycle inventory (LCI). The farmer can also use the data sharing platform to share the scan's results and (in the future) export part of the collected LCI data to other digital tools. The LCI is designed to make maximum use of standardised terminology, to make data available for other applications. To take full advantage of the benefits of data sharing platforms, consistent use of data standards and farmer acceptance remain important challenges that need to be addressed. At the moment, few data sources use standardised terminology, hampering the easy exchange of data between tools. Besides this, many Flemish farmers are still hesitant to trust and use data sharing platforms. Information campaigns and success cases like the climate scan can boost this lack of confidence and raise awareness for the benefits of safe data sharing.

#### **5.04.P-Tu395 Industry LCA for Hand Dish Washing at Colder Temperatures and Using Shorter Dishwasher Cycles**

*Jasmin Martinez, Procter & Gamble, Belgium*

Procter & Gamble's 2030 Sustainability Goals include 100% recyclable packaging, cutting sites greenhouse gas (GHG) emissions in half vs. 2010 and enabling responsible consumption for leadership brands. Encouraging consumers to use products under low-energy conditions can result in significant environmental savings, considering that 5 billion people use P&G products every day and for specific categories such as dishwashing detergent, the consumer use-phase dominates the overall life cycle impact.



This study presents the results of an ISO compliant and externally reviewed life cycle assessment for washing dishes manually at colder temperatures (23°C vs. 40°C) and for selecting a short cycle when using a dishwasher instead of the normal cycle. The main current P&G manual and machine dishwashing detergents were selected for this cradle to grave LCA. For the consumer in-use phase, 12 European countries are included and recent consumer tests used for realistic habits & practice data. The LCA results confirm the importance of the use-phase with respect to the entire dishwashing life cycle. Both for manual and machine dishwashing, more than 70% of the GHG emissions come from the consumer use life cycle step. The main conclusion of this LCA is that washing dishes by hand at colder temperatures or selecting a short instead of normal dishwasher cycle have a significant environmental benefit in all relevant impact areas, with no trade-offs. Moving from current hand dishwashing temperatures (around 40°C) to a 23°C target has the potential to reduce GHG emissions and energy consumption by more than 45% in all the European countries studied. Selecting a short vs. normal dishwasher cycle has the potential to reduce GHG emissions and energy by more than 20% and 35% respectively. Both for hand and automatic dishwashing, when the consumer use-phase is excluded, the life cycle stages that contribute mostly to climate change are the formulation (>65% for ingredients production & wastewater treatment), the packaging production & end of life (>2%) and the transportation (>2%). Manufacturing accounts for less than 2%. Overall, this ISO compliant LCA can be used to guide sustainable R&D product and packaging innovations, make business decisions, create an opportunity for environmental claims and encourage consumers to adopt more sustainable habits by using dishwashing products under low-energy use conditions without compromising on cleaning performance.

#### **5.04.P-Tu396 Development of an Automated System for Conducting Life Cycle Assessments--A Chemical Industry Example**

*Ravinder Menon, EHS, Afton Chemical*

Afton Chemical Corporation is a manufacturer of lubricant and fuel additives. About eleven years or so ago, we started getting requests from our customers for life cycle assessment (LCA) of our products and in particular, for the product carbon footprint (PCF). These requests started surging since 2019, as a consequence of industry in general and our customers in particular, being more aware of global requirements and initiatives on emissions reduction, carbon neutrality and net zero. Many were also publishing their corporate goals in this area. Afton initially responded to these requests by a combination of consultants and training. As the requests started increasing and, in some cases, providing PCF data became a requirement to bid on new business, we realized we had to change our approach from a "one off" LCA contracted to an external consultant, to the development of a more integrated and automated tool that we could deploy internally in an efficient manner.

We brainstormed the idea internally and then with our consultant. We knew this was not going to be an easy task given the complexity of our product portfolio, the large number of manufacturing sites spread globally, the large number of raw materials that go into making those products and associated complexity of various transportation modes and distances. Further complicating this issue was the fact that our internal data systems were not all on the same platform and in some cases, were manually maintained. Even the consultant, who had a lot of expertise with LCA, had not attempted anything of this complexity and magnitude before.

After several meetings between the two teams, we decided to run a pilot project, with a limited number of products covering multiple sites. The results of the pilot in 2020 showed that it was feasible to develop an integrated and automated tool for conducting LCA of our products. However, there was considerable work involved to gather data on product formulations, raw materials, inbound transportation. As far as production-related activities and emissions go, most of our sites don't have sub-metering, so an allocation methodology had to be developed. After months of work, the LCAT (Life Cycle assessment Automation Tool) system was developed. This presentation will detail the various steps in the process and how we deploy LCAT currently to meet our customer requirements and to set corporate decarbonization goals.

#### **5.04.P-Tu397 The Choice of Refractories for Steel Ladle Lining: A Life Cycle Perspective**

*Md Jubayed<sup>1</sup>, Rinus Siebring<sup>2</sup> and Angélique Léonard<sup>3</sup>, (1)Chemical Engineering, University of Liege, Belgium, (2)Ceramics Research Centre, Tata Steel, Netherlands, (3)PEPs, Laboratory of Chemical Engineering, Department of Applied Chemistry, University of Liège, Belgium*

The Steel Industry is the biggest consumer of refractories, with more than 70% of total refractory consumption worldwide. Efficient and innovative use of refractory in steel ladle lining is absolutely relevant to decarbonize the steel industry. Life cycle assessment (LCA) methodology is widely used to evaluate the environmental footprint (EF) of a product or process. However, in literature, very few LCA studies are found regarding refractories used in steel ladle, mostly focusing on manufacturing followed by end of life recycling while ignoring the operational feature (use phase) of the refractory. It is not all about embodied impacts of refractories that should be taken into account but also its properties (i.e. density, thermal conductivity and strength) and design of refractory lining which have significant effect on steel production volume and energy efficiency of steel ladle processes. Therefore, this study aims to evaluate how the choice of refractories considering its operational feature in steel ladle lining influence the overall EF of steel. Cradle-to-gate (including recycling of refractories) attributional LCA will be conducted to quantify environmental impacts of steel maintaining a consistent system boundary. Various scenarios will be analyzed using alternative refractories at each segment of the steel ladle as well as changing the thickness of refractory lining. Data will be collected from industrial partner: Tata Steel, Netherlands. The study will provide a comprehensive LCA framework to support the choice of refractories for steel ladle lining, and the case study itself will be an excellent contribution to fulfilling the current knowledge gap in the literature.

#### **5.04.P-Tu398 Use of Life Cycle Impact Assessment to Advance Holistic Optimization of Radiological Protection and Safety**

**Bryanna D Wattier**, *Lindsay C Shuller-Nickles, Michael Carbajales-Dale and Nicole E Martinez, Environmental Engineering and Earth Sciences, Clemson University*

Radiological protection seeks to protect patients, the environment, and members of the public from detrimental effects of unnecessary exposure to ionizing radiation. Within the system of radiological protection is the process of optimization, which is a cyclical process aimed at keeping the likelihood of incurring exposure, magnitude of individual doses, and the number of people exposed as low as reasonably achievable (ALARA). In order to do this, the situation is evaluated, the protective options are identified, the best option for protection is selected, and the option is implemented. Life cycle assessment (LCA) is a modeling technique that can be used to determine the cradle-to-grave impacts to humans and the environment for the production of a good or service. LCA can be incorporated into the optimization of radiation protection as a decision-aiding technique (DAT). DATs such as cost-benefit analysis and value of statistical life are already in use for radiation protection because the dose rate should not be the only factor considered for protection. Tools like LCA may be implemented into radiological protection to yield a more holistic perspective and enable comparison with analyses of non-radiological systems. Despite structural similarities to a decision-aiding technique, life cycle impact assessment (LCIA) is not commonly used in the context of optimization of radiation protection.

A proof of principle using concrete as a shielding material for a cesium-137 (Cs-137) point source was conducted for the production of concrete with different cement compositions. The concrete containing the cement composition of 21-35% limestone had the largest global warming potential and water consumption in addition to the second highest ionizing radiation emitted in the production, surpassed only by 36-55% pozzolana and fly ash cement. The concrete containing 21-35% alternative constituents cement had the lowest ionizing radiation emitted and water consumed in addition to the second lowest global warming potential, followed only by the concrete containing 36-55% pozzolana and fly ash cement. LCIA as a DAT would recommend using concrete with 21-35% alternative constituent cement. Additional DATs or impact categories could aid in the decision for the best concrete option to use for shielding the Cs-137 source.

#### **5.04.P-Tu399 Teaching Life Cycle Assessment Using Counterintuitive Examples**

**Andrea Hicks**, *University of Wisconsin, Madison*

Life cycle assessment is a fundamental tool in industrial ecology. Teaching students at a university level the skill of life cycle assessment is often accomplished through project based learning. The inherent uncertainties present in project based learning has been shown to enhance student learning. This work reflects on a graduate level course on life cycle assessment which utilizes counter intuitive examples, which are problems and systems which seem like one choice should be the clear and easy winner, but that is often not the case. In particular, using the case study on single use versus reusable drinking straws will be explored. The student's level of comfort with their knowledge was gauged with pre and post course knowledge probes, coupled with a guided reflection. This work is still in progress, but is anticipated to illustrate that the usage of counter intuitive examples causes students to probe more deeply into their preconceptions of sustainability and environmental impact. As the skill of conducting a life cycle assessment becomes a more popular and desired skill, it is critical to improve how we teach these skills.

#### **5.04.V LCA as an Effective Tool in Decision-making**

##### **5.04.V-01 Carbon footprint of Osaka Expo in 2025**

**Ryusei Murata** and *Norihiro Itsubo, Tokyo City University, Japan*

In October 2020, former Prime Minister Suga declared Japan's goal towards carbon neutrality in 2050, and in June 2021, the Green Growth Strategy in conjunction with Carbon Neutrality in 2050 was formulated. Based on goal and strategy, carbon neutrality is considered important for the 2025 Osaka Expo. In addition, the Exposition Association organizing the Expo has compiled the results of studies conducted by the Environment and Energy Committee in the Future Society as the , which sets out six directions for decarbonisation and resource recycling-related initiatives for the said event.

A previous study on Expo Milano 2015 quantifying greenhouse gas (GHG) emissions has been conducted. The main contributors in the previous study were the organisers (offices and transfers, construction sites, pavilion construction, preparatory events, exhibition information and decommissioning) and tourist transportation and movement. The overall GHG emissions for Expo Milano is approximately 103 million t-CO<sub>2</sub>eq, of which majority of the emissions were related to tourist transportation and movements accounting for approximately 60% of the total. Previous studies of large-scale events have shown a significant environmental impact related to the movement of tourists and visitors.

Based on the above, the objectives of this study are (i) to estimate the GHG emissions of the Osaka Expo in 2025. (ii) Calculate the GHG emissions of a large-scale event and (iii) present specific reduction measures, which leads to a change in people's behaviour and environmental measures.

This study also covers the Hanazono Expo, a sub-event of 2025 Osaka Expo, and the entire 2025 Osaka Expo. The GHG emissions of the sub-events are assessed by multiplying the monetary data obtained through interviews by the intensity of the Environmental Impact Intensity Data Book (3EID) based on the Japanese input-output table. The GHG emissions for the entire 2025 Osaka Expo are estimated by multiplying the GHG emissions by the intensity of the Environmental Impact Intensity Data Book (3EID) based on the inter-industry relations table from the data on the economic spillover effects of the 2025 Osaka Expo in the Kansai Economic White Paper 2022.

For future research and studies, the evaluation of sub-events and the assessment of GHG emissions from Osaka Expo 2025 Osaka should be further evaluated in detail.

#### **5.04.V-02 Life Cycle Assessment of a District Cooling Plant – Effect of Temporally-resolved Electricity and Heat Supplies**

**Doris Rixrath<sup>1</sup>, Gerhard Piringer<sup>2</sup>, Raphael Schauer<sup>3</sup> and Rosa Weber<sup>3</sup>, (1)JRZ LiSA, University of Applied Sciences Burgenland, Austria, (2)Energy and Environment, University of Applied Sciences Burgenland, Austria, (3)University of Applied Sciences Burgenland, Austria**

District cooling networks are becoming increasingly popular as a way to meet cooling demand in urban areas. Their environmental impact is a fundamental consideration. Typically, life cycle assessments (LCA) results for cooling devices are based on annual average electricity and heat mixes. However, electricity and heat mixes in Austria exhibit both seasonal and daily fluctuations. The objective of this study is (a) to investigate the life cycle environmental impacts of operating a district cooling plant in Vienna, and (b) to study the effect on LCA result of an hourly resolution of both the plant's energy supply (electricity and heat mix) and of its cooling load profile, relative to an annual average resolution.

This study is an attributional LCA of a 19.8-MW district cooling plant featuring four electrically-powered compression chiller units, as well as two district-heat supplied absorption chiller units, and one free cooling system. The Hourly load profile data for the cooling demand were provided by the local utility for the year 2019 and used to weigh the hourly electricity and heat mixes that supply the chiller units. Hourly electricity mix data were obtained for the national grid of Austria in 2019 [4], and hourly heat mix data for district heat for the absorption chillers were supplied by the local utility. The functional unit for the LCA was 1 kWh of cooling energy.

LCA results for the cooling plant will be presented for the individual chiller units as well as for entire cooling plant. The findings will analyze and compare the impacts of the individual different chillers, and compare these impacts to those derived from average annual supply mixes. Preliminary results indicate that load-induced differences in the supply mixes between individual units are as large as the differences to the average annual supply mixes.

The plant's impact is expected to be dominated by the operating energy supplies. Based on previous research on a heat pump, we further expect that the hourly-resolved impacts will clearly differ from those calculated with the annual average mix.

A higher temporal resolution should have a moderate but discernible effect on the LCA of operating a district cooling plant, particularly in a country such as Austria that has a high share of renewables in its electricity and heat mixes. Therefore, these results will form an improved basis for decision-making by urban energy managers.

#### **5.04.V-03 The Environmental Cost of Plant-based Meat: A Lifecycle Assessment**

**Nichole Eunice Yanson Lalas<sup>1</sup> and Norihiro Itsubo<sup>2</sup>, (1)Department of Environmental Management and Information Studies, Tokyo City University, Japan, (2)Tokyo City University, Japan**

The growing global demand for beef brings environmental consequences of deforestation, climate change, and waste disposal issues. Several businesses today have been innovating toward producing alternative meat to lessen the industry's environmental impact without compromising economic profitability. With consumers gaining awareness of responsible consumption, market demand for alternative meat is booming. Businesses are developing different technologies to meet this demand. Shifting from conventional meat to plant-based meat also aligns with the UN's Sustainable Development Goals on Zero Hunger, Responsible Consumption and Production, Climate Change, and Life on Land.

This study aims to determine the environmental impact of plant-based and conventional meat. Other objectives include the determination of which alternative meat and which specific product lifecycle process contribute the lowest and highest impact. The study will aid in providing insights and recommendations to assist businesses in strategizing their environmental and economic sustainability initiatives, to aid the government in establishing feasible and practical solutions for future policymaking, to promote individuals' responsible consumption, to help farmers better take care of their farm fields, and to maintain a clean, healthy, and green environment.

The main research subject is DAIZ, Inc, a company producing and selling germinated soybeans and peas in Japan which will then be compared to conventional red meat based on existing literature. The system boundary will cover from cradle (accounts land use change)-to-consumer approach.

Environmental impact is measured through Lifecycle Assessment.

The study will utilize SimaPro 9 software and EcoInvent, IDEA, and openLCA databases. Primary data for plant-based meat and secondary data for conventional meat will be sourced from DAIZ and existing literature.

Expected results of the study include the Environmental Impact of Conventional and Plant-based Meat as measured through GHG emissions and comparative analysis to determine the key differences between output results.

Future research includes the comparative study on DAIZ's germinated soybean and pea plant-based meat versus Impossible Meat, Beyond Burger, and Cultured Meat and the application of the Lifecycle Costing and Social Lifecycle Assessment.

#### **5.05 Life Cycle Impact Assessment Modeling and Application**

##### **5.05.P-We330 Ecotoxicity Impact Evaluation for Data Poor Chemicals Under the Global Life Cycle Impact Assessment Method Framework**

**Melanie Douziech<sup>1</sup>, Mikolaj Owsianiak<sup>2</sup>, Laura Golsteijn<sup>3</sup>, Rosalie van Zelm<sup>4</sup>, Susan A Oginah<sup>2</sup>, Olivier Jolliet<sup>2</sup>, Michael Z. Hauschild<sup>2</sup>, Leo Posthuma<sup>5</sup> and Peter Fantke<sup>2</sup>, (1)Agroscope, Switzerland, (2)Technical University of Denmark, Denmark, (3)PRé Sustainability, Netherlands, (4)Department of Environmental Science, Radboud Institute of Biological and Environmental Science, Radboud University, Netherlands, (5)National Institute for Public Health and the Environment (RIVM), Netherlands**

Recommendations from the Global Life Cycle Impact Assessment (GLAM) initiative for ecotoxicity include deriving characterization factors based on measured effect concentrations where 10% of the tested organisms show an effect (EC10) for at

least five species from three species groups. Chemicals with lacking or few measured effect data can therefore not be characterized. One aim of the third phase of the GLAM initiative is to propose practical guidelines for applying the framework to data poor chemicals. To reach this aim, we systematically compare HC20, hazardous concentration, where 20% of the species are exposed above their chronic EC10 derived from modeling approaches, against those obtained from measured effect data. Investigated modelling approaches include extrapolation methods, such as acute-to-chronic ratios and interspecies correlation estimation equations (ICE), and pure prediction tools estimating effect data from physico-chemical properties of the analyzed chemicals (Quantitative Structure-Activity Relationships, QSARs). Based on different criteria, we chose the consensus models of T.E.S.T. to estimate EC50 for *Fathead minnow* and *Daphnia magna* and the VEGA Combase QSAR for the EC50 of *Raphidocelis subcapitata*. Comparing HC20 based only on measured chronic EC10 to HC20 based on a combination of measured chronic EC10 and extrapolated ones led to an  $R^2$  of 88%. Together with already published studies, this supports the use of extrapolation methods whenever measured chronic EC10 are missing. Comparing HC20 relying only on measured, potentially extrapolated, effect data for at least five species from three species groups with HC20 including all measured algae effect data and combinations of two measured and all ICE-based effect data for the missing species group led to  $R^2$  around 50%. The  $R^2$  increased to 63% if all measured effect data were used. Finally, preliminary results showed that completing a set of four measured effect data, meaning two effect data per species group, with one QSAR estimate of the missing species group lead to  $R^2$  above 65%. Based on these findings and published results from the scientific literature, extrapolation methods might be mature enough to help characterize the ecotoxicity of data poor chemicals. Research is ongoing to formulate clear recommendations and evaluate the usefulness of pure prediction tools also in combination with extrapolation methods, particularly for chemicals with no measured effect data.

#### 5.05.P-We331 Deriving Global Normalization References Based on Unit Process Databases

**Nils Thonemann<sup>1</sup>** and **Alexis Laurent<sup>2</sup>**, (1)Danish Technical University, Denmark, (2)Technical University of Denmark, Denmark

According to ISO 14044, normalization is an optional step when conducting a life cycle assessment (LCA) and relates characterized life cycle impact assessment results with a reference. The used data source for building such inventories may differ substantially. Deriving consistent global inventories for all impact categories remains a challenging task due to (i) data availability (e.g., production amount unknown), (ii) emission coverage (e.g., emission not covered by emission report), (iii) inconsistency in data sources (e.g., different data sources and quality for climate change-related and acidification-related emissions), and (iv) temporal mismatch (e.g., outdated reference year). Until now, published normalization references are derived from different data sources. Due to these uncertainty sources and limitations, current LCA practice often leads to normalized impact profiles that are distorted or biased for some impact categories. This limits the LCA practitioners in the interpretation of their normalized impact results. To overcome the outlined challenges, we propose a new approach to derive global normalization references based on unit process databases that address some of the aforementioned problems. This approach allows for the consistent derivation of global normalization references while considering (i) production volumes from one source, (ii) covering emissions as in the background system of the modeled system under study in an LCA, (iii) using the same data source for all data, and (iv) allowing for annual updates. This work has been conducted in the Working Group on Normalization within the Task Force for cross-cutting issues in life cycle impact assessment (LCIA) under the flagship project "Global Guidance on LCIA Indicators and Methods" (GLAM). The overall approach considered in this work is the exploitation of life cycle inventory databases and production data to calculate normalization references. With the described approach, globally consistent normalization references could be derived. To check the procedure's validity, we compared the newly calculated normalization inventories with former normalization inventories. The coefficient of determination ( $r$ -square), which was calculated to be 0.72, is considered acceptable and unveils for greenhouse gases that the approach using process databases can produce normalization inventories similar to established normalization inventories.

#### 5.05.P-We333 Is Adoption of Agroforestry in Arable Systems Environmentally Sustainable? Accounting for Ecosystem Services in Life Cycle Assessment

**Lieslot Boone<sup>1</sup>**, **Paul Pardon<sup>2</sup>**, **Bert Reubens<sup>2</sup>**, **Kris Verheyen<sup>3</sup>** and **Jo Dewulf<sup>1</sup>**, (1)Green Chemistry and Technology, Ghent University, Belgium, (2)Flanders Research Institute for Agriculture, Fisheries and Food, Belgium, (3)Environment, Ghent University, Belgium

Intensive agricultural management, despite higher crop productivity, also led to environmental concerns such as biodiversity loss or eutrophication. This gave rise to calls for alternative systems, such as agroforestry, where the delivery of food and biomass, i.e. provisioning ecosystem services ( $ES_{prov}$ ), is balanced with the supply of regulating ( $ES_{reg}$ ) (e.g. erosion prevention) and cultural (e.g. recreation) ES. But agroforestry can lead to lower crop yield due to effects of tree presence. Therefore, analysing the environmental sustainability by e.g. life cycle assessment (LCA), including all ES supplied, is required. Though LCA traditionally only considers the harvested product, whereas the product provided by an agricultural system actually includes a bundle of ES. To address this issue, LCA is here extended with ES assessment to compare the resource footprint of two conventional systems: a field with only an arable component (pure conventional system) and a field (same size) with the same arable crops but with also a tree component (conventional agroforestry system). To distribute the environmental impact across all agricultural output, i.e.  $ES_{prov}$  and other ES, allocation factors are calculated. They are defined based on the capacity of the two ecosystems to deliver specific ES. These ES data comes from measurements in Flanders (Belgium) that resulted in a long-term dataset. A weighting factor  $\alpha$  (ratio of  $ES_{reg}$  over  $ES_{prov}$ ) is introduced to account for the importance of the bundle of  $ES_{reg}$  relative to  $ES_{prov}$ . If only  $ES_{prov}$  are considered, the pure conventional system has a 4 or 15% lower resource footprint than agroforestry, per kg dry matter or per euro of harvested biomass, respectively. However, at equal weighting of  $ES_{prov}$  and  $ES_{reg}$  (i.e.  $\alpha = 1$ ), agroforestry is preferred to the conventional system, with the latter having an 11 (mass) or 1% (economy) higher footprint. The tipping point at

which agroforestry is less resource-intensive than the pure conventional is at 0.14 (impact per kg) or 0.87 (impact per euro). This indicates the importance of expressing the impact in both mass and economic values and opens the interest in further research into a functional unit that is independent of economic fluctuations but includes the value of harvested products. The new approach including ES assessment enables thus for a more comprehensive comparison of the environmental sustainability of agricultural systems.

#### **5.05.T-01 Characterizing Sea Level Rise Potential Impacts on Coastal Ecosystem Services in Life Cycle Impact Assessment**

**Laura Debarre<sup>1</sup>, Manuele Margni<sup>1</sup> and Martin Dorber<sup>2</sup>, (1)CIRAIG - Ecole Polytechnique de Montreal, Canada, (2)Norwegian University of Science & Technology, Norway**

Oceans and coastal ecosystems provide many “ecosystem services” (ES) to humans including food production, climate regulation or coastal protection. Sea level rise (SLR) due to climate change will have a significant influence on coastal ecosystem functions and services due to changes in habitats and species communities. There is growing support for integrating the potential impact of human activities on the functional value of ecosystems as new Area of Protection into the Life Cycle Assessment (LCA) framework. Nonetheless, current coverage of anthropogenic potential impacts on ES in LCA is limited. This research proposes characterization models and factors assessing the potential cost to society resulting from a change of potential coastal ES supply triggered by climate-change-driven SLR. This work offers an innovative approach to integrate ES within LCIA framework building on an ES matrix approach and the benefit-transfer method for the monetary valuation of ES. This method builds on the assessment of the potential ES value of habitats likely to be inundated due to climate-change-driven SLR under two different GHG emission scenarios. Characterization factors (CFs), expressed in \$/tCO<sub>2</sub>, represent an average global potential impact for RCP4.5 and RCP8.5. These CFs account for the cumulative loss in value of the ES supply between 2015 and 2100 divided by the cumulative amount of CO<sub>2</sub> emitted over the same period. The mapping and identification of habitats under threat of inundation relies on global maps of present (year 2015) and projected (2050 and 2100) inundated coastal zones due to SLR. These inundation maps are coupled with global mappings of Ecosystem Functional Groups (EFG) defined as the main reporting unit for ES, to identify the ES that are present in the inundated zones. The annual monetary value of those ES (in \$/yr) is estimated building on the Ecosystem Service Valuation Database. Results show that the global value of areas likely to be inundated due to SLR, in terms of potential ecosystem services provision, increases by \$1.91E+14 (\$2.47E+14 respectively) between 2015 and 2100 under the RCP4.5 (RCP8.5). CF were found to reach 72.1 (35.9) \$/tCO<sub>2</sub> for RCP4.5 (RCP8.5). Values are probably overestimated and should be reduced by the residual value of the EFG to which the ecosystem is transitioning. Results suggest that potential SLR impacts on coastal ecosystems are significant, highlighting a need for further research on this topic.

#### **5.05.T-02 Estimation of Potential Economic Value Losses Due To Resource Dissipation in LCA Through The EVDP Method: A Case on LIB Recycling**

**Jair Santillán-Saldivar<sup>1</sup>, Antoine Beylot<sup>2</sup>, Stéphanie Muller<sup>3</sup>, Emmanuelle Cor<sup>4</sup> and Elise Monnier<sup>4</sup>, (1)University of Bordeaux, France, (2)French Geological Survey, France, (3)Bureau de recherches géologiques et minières BRGM, France, (4)CEA-Commissariat à l'Energie Atomique et aux énergies Alternatives, France**

Resource dissipation is a phenomenon that has been identified as a barrier towards circular economy causing losses in the technosphere along the life cycle of products. When addressing mineral resources, an identified safeguard subject is the potential to make use of the value that a resource holds for humans; therefore a damage would be the reduction or loss of this potential due to human activity; currently there is a limited number of proposals to assess the impacts of dissipative losses.

Building upon the JRC-LCI and JRC-LCIA methods, we present the Economic Value Dissipation Potential (EVDP) method, an impact assessment method in LCA that integrates the identification of dissipative flows, and the potential value loss associated to them. The method proposes a function that estimates the fraction of a mass flow that can be considered dissipative based on the comparison between a global average minimum grade in primary sources and resource concentration in dissipation compartments. Next, the method assigns a value to this flows based on the integration of their price and economic importance to model a potential value loss.

The method is applied to a case study on Lithium-ion batteries (LIB) recycling through hydrometallurgy. Partial results of the application of the EVDP method include the development of a resource flow analysis based on the JRC-LCI method to better trace the analyzed materials. Results show that the recycling of a kilogram of battery NMC 111 allows the avoidance of the equivalent to 3.79 USD in dissipative losses. The biggest contributors being cobalt due to its high price and economic importance from the point of view of the EU, and aluminum, due to being the element with greater recovered mass in the analyzed process. The EVDP method provides a way to associate potentially dissipative flows to value losses measured in monetary units. It also provides an indicator to quantify the effectiveness of circular economy strategies in maintaining the value of resources in the technosphere. The application of the method to a case study on LIB recycling through hydrometallurgy shows the benefits of this type of practices from a life cycle perspective; further replication of the method is suggested along with other impact assessment methods to support decision-making processes regarding the implementation of circular economy strategies.

#### **5.05.T-03 Temporal Inconsistencies in LCA: Dynamic Climate Change Characterization Factors**

**Guillaume Batôt, François-Xavier Dezert, Sibylle Duval-Dachary and Sandra Beauchet, IFP Energies Nouvelles, France**

Achieving Net Zero by 2050 will notably induce delayed emissions, for instance due to carbon capture and utilisation (CCU), e.g. emissions optimisation through temporary storage in materials. The effects of temporary storage on climate change could be worse than doing nothing. That is why impacts should be carefully assessed using dynamic life cycle assessment (D-LCA). In such a rapidly evolving context, considering temporality in LCA is becoming more and more crucial.

The purpose of this paper is to review the dynamic GWP indicator based on the "time corrected" approach initially proposed by Levasseur, and recently revised by Ventura with the "fixed horizon of impact" method. Even though the latter are useful and provide a clear improvement compared to the basic use of a static indicator in D-LCA, both solutions may not seem entirely satisfactory and a modified version is suggested to keep what seems relevant in each of these two approaches. These different dynamic versions of the GWP/GTP indicator are compared with regard to the static indicator. Three temporal parameters are therefore used: the time horizon of the study, indicating the total time over which impacts are effectively assessed; the life cycle duration, which is the period of time between the first and the last inventory flow; and the time horizon of the impact, defined by the practitioner (e.g. usually 100 years for the GWP). Applied to the case of a simplified CCU system (life cycle duration of 45 years), the "time corrected" approach and the "fixed horizon of impact" method give similar values and trends for the GWP and GTP, when the time horizon of impact is greater than 20 years. The modification introduced in this work leads to opposite trends and in particular gives higher importance to a future increase in global temperature – that will actually be felt by the next generations. The choice of the temporal method for calculating a "dynamic" impact is not neutral and should be considered and discussed depending on the stakes at hand. Moreover, adopting a cost-benefit viewpoint would allow to interpret the trends as the weight given to future generation with regards to the actual one: a more than central issue today.

## 5.05.P Life Cycle Impact Assessment Modeling and Application

### 5.05.P-We332 Path dependency in Life Cycle Impact Assessment: Are All the Impact Categories Still Relevant?

**Jan Matušík**, *Department of Sustainability and Product Ecology, University of Chemistry and Technology Prague, Czech Republic*

Life Cycle Assessment can boast quite a long and rather successful history, being now established at the highest policy levels. The methodology has been evolving for over 30 years, and arguably reached levels of maturity allowing it to be utilized as an official assessment tool, as manifested in the EU Product Environmental Footprint. The EF methodology provides a subset of recommended impact categories, which is in essence similar to the ensemble first used in the CML methodology from the 1990s. The assessed impact categories should be selected to represent the most pressing environmental issues, i.e., they should reflect what society (researchers) perceives as the most relevant. Nevertheless, the environmental debate keeps on evolving: from the focus on radioactivity, through chemical pollution of substances like DDT, resource depletion, forest die-off caused by acidification, or ozone depletion at the end of 1980s, to the current issues of climate crisis, biodiversity destruction, or plastic pollution. In the 1990s, when the subset of impact categories still in use was developed, some environmental issues were well on the road to being "solved" (e.g., acidification in Europe or stratospheric ozone depletion), while some issues were not even formulated or envisaged (ocean acidification or microplastic pollution). While new impact assessment methodologies targeting "emerging" issues are being developed, most LCAs employ the traditional set of categories. Another potential problem with the traditional set of impact categories is that some issues are overrepresented (in Environmental Footprint there are three categories focusing on eutrophication). It begs to question whether this is an intended manifestation of the perceived import or an "accident". Another long-debated conundrum is the issue of resource depletion, which is a purely socio-economic problem. Clearly, the commonly used subset of impact categories is a residuum of a past worldview. As most complex systems, the evolution of impact assessment methodology is path-dependent, the decisions of past researchers still greatly affect how we do LCA today – while many different paths could have been taken just as well. Nevertheless, from a position of a person getting into LCA later, some of those decisions do not seem to make sense anymore. It might be time to reassess which impact categories are still relevant, and which environmental issues are the most pressing in today's world.

### 5.05.P-We333 Is Adoption of Agroforestry in Arable Systems Environmentally Sustainable? Accounting for Ecosystem Services in Life Cycle Assessment

**Lieslot Boone<sup>1</sup>**, **Paul Pardon<sup>2</sup>**, **Bert Reubens<sup>2</sup>**, **Kris Verheyen<sup>3</sup>** and **Jo Dewulf<sup>1</sup>**, *(1)Green Chemistry and Technology, Ghent University, Belgium, (2)Flanders Research Institute for Agriculture, Fisheries and Food, Belgium, (3)Environment, Ghent University, Belgium*

Intensive agricultural management, despite higher crop productivity, also led to environmental concerns such as biodiversity loss or eutrophication. This gave rise to calls for alternative systems, such as agroforestry, where the delivery of food and biomass, i.e. provisioning ecosystem services ( $ES_{prov}$ ), is balanced with the supply of regulating ( $ES_{reg}$ ) (e.g. erosion prevention) and cultural (e.g. recreation) ES. But agroforestry can lead to lower crop yield due to effects of tree presence. Therefore, analysing the environmental sustainability by e.g. life cycle assessment (LCA), including all ES supplied, is required. Though LCA traditionally only considers the harvested product, whereas the product provided by an agricultural system actually includes a bundle of ES. To address this issue, LCA is here extended with ES assessment to compare the resource footprint of two conventional systems: a field with only an arable component (pure conventional system) and a field (same size) with the same arable crops but with also a tree component (conventional agroforestry system). To distribute the environmental impact across all agricultural output, i.e.  $ES_{prov}$  and other ES, allocation factors are calculated. They are defined based on the capacity of the two ecosystems to deliver specific ES. These ES data comes from measurements in Flanders (Belgium) that resulted in a long-term dataset. A weighting factor  $\alpha$  (ratio of  $ES_{reg}$  over  $ES_{prov}$ ) is introduced to account for the importance of the bundle of  $ES_{reg}$  relative to  $ES_{prov}$ . If only  $ES_{prov}$  are considered, the pure conventional system has a 4 or 15% lower resource footprint than agroforestry, per kg dry matter or per euro of harvested biomass, respectively. However, at equal weighting of  $ES_{prov}$  and  $ES_{reg}$  (i.e.  $\alpha = 1$ ), agroforestry is preferred to the conventional system, with the latter having an 11 (mass) or 1% (economy) higher footprint. The tipping point at which agroforestry is less resource-intensive than the pure conventional is at 0.14 (impact per kg) or 0.87 (impact per euro). This

indicates the importance of expressing the impact in both mass and economic values and opens the interest in further research into a functional unit that is independent of economic fluctuations but includes the value of harvested products. The new approach including ES assessment enables thus for a more comprehensive comparison of the environmental sustainability of agricultural systems.

#### **5.05.P-We334 Development of Biodiversity Damage Assessment in LCIA with the Expanded Impact Categories**

**Runya Liu<sup>1</sup>, Haruka Ohashi<sup>2</sup>, Akiko Hirata<sup>2</sup>, Tetsuya Matsui<sup>2</sup> and Norihiro Itsubo<sup>3</sup>**, (1)Graduate School of Environmental and Information Studies, Tokyo City University, Japan, (2)Forestry and Forest Products Research Institute, Japan, (3)Tokyo City University, Japan

During the past century, our planet has gone through an unprecedented change, which boosts human society's development rapidly. However, the alarm of entering the sixth mass extinction urges us to reconsider the necessity of human-nature balance. As IUCN is now working on developing a quantitative methodology to measure and track contributions toward protecting and restoring nature, which is aiming at contributing to a nature-positive future. Lacking a powerful and comprehensive methodology may prevent biodiversity restoration and protection.

Therefore, we are refining the Life cycle impact assessment (LCIA) methodology to offer a far-reaching way to well-consider biodiversity even the implementation by policymakers and stakeholders.

In this research, we have three main objectives. First, try to refresh the LCIA midpoint category menu which is considered to affect biodiversity. Second, compare the LCIA biodiversity indicators and clarify the scientific performance of each, the Potentially disappeared fraction (PDF), and the Expected Increase in the Number of Extinct Species (EINES). Last, try to include the species in the evaluation as much as possible.

As the methodology, by referring to the Life cycle Impact assessment Method based on the Endpoint model 3(LIME3) developed framework, we lodge the biodiversity loss at the stage of the endpoint. Then we integrate the LIME3 framework with Max Entropy (Maxent) which is an application of the species distribution model (SDM). Finally, the output from the model could be applied with LCIA indicators in procuring the damage factors.

As the result, we modified the PDF and compared it with EINES, and the original PDF. Some significant limitations could be eliminated and improved. Then we applied it try to view the global biodiversity status. We found that the mean PDF (Current ~RCP8.5 scenario) shows the highest in the European region and lowest in Asia. The Amphibia shows the highest risk compare to another taxon; the plant is next to it. Bird is most flexible under this scenario due to the long-migration ability.

In future work, we are pursuing to make win-win collaborative research on LCIA and ecology. Borden the possibility to implement the ecological knowledge by LCA users even the business. On another side, gain more agreement on methodology and indicators from a nature-based evaluation. Hope to further contribute to the nature-positive system.

#### **5.05.P-We335 Developing the Fisheries Impact Pathway - Operationalised in the Context of GLAM Phase 3**

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Marine biodiversity impact coverage is still scarce in the current Life Cycle Impact Assessment (LCIA) framework. The work presented in this abstract supports the development and operationalisation of the recently proposed impact pathway for fisheries impacts on ecosystem quality. This is also a subject of discussion in GLAM Phase 3.

Updated Characterisation Factors (CFs) have been computed, based on a popular fisheries stock management model based.

Objectives include incorporation of discards from by-catch in the impact quantification, and to explore the temporal validity of CFs relative to fisheries' trends.

CFs have been computed from depleted fractions of individual stocks (a species in a habitat) and regionalised at the scale of FAO major fishing area. Points of development include: 1) Input data updated from 2015 to 2018, enabling determination of questions of temporality in static CFs. 2) Inclusion of additional but "invisible" impact of discards in fishing activity within the impact assessment. 3) Regional to global impact conversion using two comparable methods, at the regional scale and a species-level alternative to attribute a measure of vulnerability related to species endemism.

Results include an updated set of operational CFs for the depletion of individual stocks, within current data constraints and with an endpoint unit converted into PDF·year/kg biomass. The CFs should be consistent with Life Cycle Initiative guidelines and could be included in GLAM Phase 3, available for use in LCA studies. The outputs of two regional to global conversion factors are compared to understand whether it is possible to have a per-stock representation of impact at the global scale to give a measure of the endemism of the impacted stock rather than an aggregation per region. The inclusion of discards increases the impact of depleting each stock by 52% on average. Due to a lack of explicit data, this first attempt is a regional estimation, with the likelihood of accidental capture and subsequent rejection based on the biomass of each species present in the region only.

The current format of the approach and CFs are highly relevant for compatibility with fisheries management regulations and show potential for inclusion in GLAM Phase 3. The inclusion of discards is an important point of progression for the fisheries impact pathway. A future perspective to further develop this impact pathway is the incorporation of ecosystem dynamics.

#### **5.05.P-We336 Ecotoxicity Impacts by the Pesticide Usage in Berry Open Field Production - A Case Study in Finland**

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Comprehensive data on the use of plant protection products (PPP) and their impacts on different berries are scarce. In this case study, we mapped the use of PPPs on four open field strawberry and three raspberry farms in Finland in 2017-2021 and calculated

their potential freshwater ecotoxic impacts using a method based on the SETAC consensus LCIA (life cycle impact assessment) model of Usetox 2.0. PestLCI Consensus V.1.0 model was used to estimate the pesticide emissions. In the model, parameters that best described a common Finnish strawberry and raspberry plant protection practices and initial primary emission distribution fractions were used. Twenty eight (28) different active ingredients (in average 1,8 kg/ha/year) on strawberry and six (in average 0,6 kg/ha/year) on raspberry were used during the studied time period, respectively. Strawberries were sprayed about 7 times and raspberries about 2 times per year (=one spray equals to use of one active ingredient). Fungicides were the most commonly used pesticides on both crops. According to the impact results, there were large variations between farms in ecotoxic pressure, depending also on the used amounts of PPPs. The impacts of strawberry production were greater than raspberry. According to the sensitivity analysis, plant growth rate and spraying method had an effect on the ecotoxic pressure. A fungicide azoxystrobin induced the greatest impact (35.2 %) of all used pesticides on strawberry (4633 PAF m<sup>3</sup> d/ha/year) and a fungicide iprodione (59.5 %) on raspberry (2058 PAF m<sup>3</sup> d/ha/year). These were followed by insecticide pyrethroid lambda-cyhalothrin and herbicide diquat on strawberry, and an insecticide pyrethroid deltamethrin and fungicide fludioxonil on raspberry. The total impact was 13148 PAF m<sup>3</sup> d/ha/year and when compared to the yield i.e. 0.40 PAF m<sup>3</sup> d/kg/year on strawberry and 3457 PAF m<sup>3</sup> d/ha/year and 0.02 PAF m<sup>3</sup> d/kg/year on raspberry. Using this LCA-based method the environmental impacts of individual hazardous PPPs can be identified. The results show that environmental impacts of PPPs can be decreased by looking for alternatives for the most harmful substances and develop the use of PPPs in a more sustainable direction. Still, extension of broadening applicability of the method needs further development.

#### **5.05.P-We337 Life Cycle Toxicity Impact on Human and Ecosystem Health Caused by Pesticides and Related Chemicals in Major Crop Production Systems in Thailand**

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Thailand is a global producer and exporter of various products from major crops. Since pesticides can harm humans and the environment, the current study aims at characterizing the life cycle toxicity impacts on humans and the ecosystem arising from pesticides and related chemical emissions in major crop production systems in Thailand. Pesticides produced and then used in Thai major crop production systems (rice, cassava, sugarcane, and oil palm) are evaluated for human toxicity and ecotoxicity. The inventories are structured into direct (pesticides applied on farms) and indirect (any toxic substance emissions in the upstream processes of pesticide manufacturing) emissions. Three assessment systems are carried out (direct impact-based PestLCI and PEFCR, and the indirect impact-based ecoinvent). Thai-specific characterization factors (CFs) of pesticides are used to yield the total direct impact scores. The indirect impact is quantified using CFs from USEtox 2.12, in SimaPro. Our results illustrate that, for human toxicity impacts, the total direct and indirect emission-related impact is 0.13 and  $4.6 \times 10^{-5}$  DALY/t. Direct emissions are mainly contributing to total human health impact scores ranging from 87 to 100%. The rice system shows the highest direct impact of 0.12 DALY/t mainly caused by Butachlor (51%). The obtained impacts based on PestLCI emissions are up to 2 orders of magnitude higher than those based on PEFCR for rice and sugarcane systems. PestLCI considers emissions reaching the field crop, which will be consumed and cause additional impacts. PEFCR does not account for crop emissions. For ecotoxicity impacts, the total direct and indirect emission-related impact is 3,806 and 104,179 PDF m<sup>3</sup> day/t. Indirect (i.e., supply chain) emissions are mainly contributing to total ecotoxicity impacts ranging from 93 to 99%. The rice system also shows the highest indirect impact of 70,332 PDF m<sup>3</sup> day/t caused by various herbicides (e.g., Alachlor (20%) and Acetochlor (20%)). The materials used in pesticide production can emit numerous toxic substances. Our study illustrates that direct emissions of pesticides are crucial to be considered in LCA due to the majority of total human health impact scores. Indirect emissions dominate the ecotoxicity impact results. The results can fluctuate depending on the underlying emission modelling approaches. Taking into account all direct and indirect emissions helps increase the accuracy of LCIA results.

#### **5.05.P-We338 Ecosystem impacts of Bio-hybrid Fuels in the LCIA: Investigation of Current Model Integration**

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Life Cycle Assessment (LCA) is an important method to estimate and compare the impacts of different substances in terms of their potential effects on humans and the environment. In the context of a complete life cycle assessment, we focus particularly on the LCIA phase. Special attention is paid to the consideration of possible impacts on the ecosystem. Initially, the objective is to obtain an adequate insight into the available LCIA models and to decide which model candidates could be used for further investigation in the next step. In addition to the general analysis, the most relevant models are compared in the context of a practical application. The characterisation factors for a bio-hybrid fuel candidate are calculated and compared in LCIA models, such as USEtox or ReCiPe. Furthermore, the models themselves and the assumptions integrated into them will be examined in more details. The aim is to investigate at a conceptual level about how to consider the impact of new potential fuel sources on the environment. In addition to data availability, the consideration of various environmentally relevant aspects will also be addressed. Overall goal is to find out which aspects of environmental effect assessment are already integrated and to what extent further aspects might need to be added into the calculated characterisation factors to sufficiently consider the potential environmental impacts. An appropriate model should be transparent and well documented. This investigation of the conceptual approach to



estimate the potential impact on ecosystems is important to uncover data gaps in the LCA and to better predict the influence of a substance release on the quality of the environment in the future. This work is intended to provide an overview of the assumptions currently used to estimate the potential impacts on ecosystems.

## 5.06.A Prospective Life Cycle Assessment of Emerging Technologies

### 5.06.A.T-01 Prospective, Anticipatory and Ex-Ante – What's the Difference? Sorting Out Concepts for Time-Related LCA

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Most life cycle assessment (LCA) studies have considered technologies as they are at the time of the study, often in a mature state. Increasingly, LCA studies attempt to assess emerging technologies in imagined states at future points in time, often referred to as prospective, anticipatory or ex-ante. However, a clear distinction between these LCA types is lacking. We aim to sort these concepts into a typology of time-related LCAs, contributing to more purposeful methodological choices. Existing frameworks for time-related LCA types were reviewed and a typology consisting of three dimensions was found to capture the most important differences. The first dimension is real time, which captures the time difference between the functional unit and the LCA. If the technology is modelled at approximately the same time as when the LCA is conducted, it can be called contemporary LCA. If the technology is modelled at a future point in time relative to the analysis, it can be called prospective LCA, and retrospective LCA if it is modelled at a past point in time relative to the study. Dynamic LCA accounts for that a technology can be “stretched out” along the real time dimension. The second dimension is technology maturity, which can be measured by technology readiness levels (TRLs). Ex-ante LCA considers technologies that are immature at the time of the study but model them in a future when they are assumed to have become mature, and is thus a specific type of prospective LCA. In contrast, ex-post LCA refers to studies of technologies that have reached maturity at the time of the study. Anticipatory LCA is effectively similar to ex-ante LCA but also entails the inclusion of numerous stakeholders in shaping the LCA study. Lab-scale LCA is a contemporary LCA of an immature technology with the aim of suggesting improvements to technology developers. The third dimension is causality. Some LCA studies mainly consider causes of a functional unit, which is often referred to as attributional LCA. Other LCA studies mainly consider effects of a functional unit, which can be called consequential LCA. While the former can be said to look backwards in time, the latter can be said to look forward in time from the perspective of the functional unit. Both types can, however, be retrospective, contemporary, or prospective LCAs as defined above. It is also possible to consider different types of causality, which relate differently to real time and technology maturity.

### 5.06.A.T-02 Prospective Life Cycle Assessments Of Chemicals: Improving Stoichiometry-based Methods

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Novel production pathways are key for the chemical industry to reach more circularity and reduce the global warming impact. To focus research and development efforts on those technologies offering the largest potential to reduce environmental impacts, prospective life cycle assessment (LCA) should be deployed already in the early stages of process development. However, the quality of input data determines the significance of the LCA results and limits their applicability to decision-making. Particularly for novel processes, knowledge is limited, forcing LCA practitioners to make assumptions and estimate missing inputs. Often, stoichiometry-based approaches are used to estimate missing chemical life cycle inventory data directly from the reaction equation. However, the accuracy of these approaches has not yet been evaluated for a wide range of chemical processes. Thus, no informed decision can be made on the best use of stoichiometry-based approaches.

In this study, we benchmark five stoichiometry-based estimation approaches with a newly derived set of 474 chemical life cycle inventories and derive suggestions for improvement. For this purpose, we compare the results of the estimation approaches to our benchmark data set. We calculate the prediction accuracy for the overall global warming impact, the global warming impact of the raw materials used, and of the process utilities. As a measure of prediction accuracy, we apply the cost estimate class system proposed by the Association for the Advancement of Cost Engineering (AACE).

Our results show that most estimation approaches underestimate the overall global warming impact for more than 70 % of all processes. As the prediction quality differs for raw materials and utilities, we identify the best-performing approach for estimating the global warming impact of raw materials and for the impact of the process energy utilities, respectively. Combining those two approaches increases the overall prediction accuracy from 73 % to 75 % in a worst-case energy scenario but from 76 % to 89 %, assuming the European grid mix.

Our results can be used to estimate more reliable inventories during research and development, improve background data, and inform decision-making by providing insights about the accuracy of the estimation approaches. As many chemical process inventories are not publicly available or roughly estimated using averaged proxies, the combined estimation approach can be applied to close these data gaps.

### 5.06.A.T-03 Prospective Life Cycle Assessment to Support Decision-making Issues: A Novel Methodological Framework

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Prospective Life Cycle Assessment (P-LCA) is a methodology that can be used to predict the environmental impact of product systems, or as future-oriented decision-making supporting tool. P-LCA models are usually based on scenarios designed by combining the foreground parameters of the model. These scenarios can be cross-compared to take the most sustainable decision among a certain range of options. This paper presents an alternative approach that is not based on a comparative assessment, but on an optimization algorithm that identifies the most sustainable solution on a mathematical basis. This approach simplifies the

scenario definition by limiting the number of foreground parameters only to *externalities*, namely those parameters that are not affected by the choice of decision-makers. On the other hand, *internalities* represent those parameters on which decision-makers can have an influence. The optimal values of *internalities* are calculated with an optimization algorithm that minimizes the environmental impact of a product system. A set of constraints shall also be defined to include correlations between all the parameters of the model. This approach has been already used in a case study whose objective is planning the future sustainable development of the automotive batteries industry in Europe. The *externalities* of the model are i) the type of batteries imported from external producers, ii) the maximum annual growth of the batteries industry, iii) the batteries demand, and iv) the percentage of wastes repurposed in second-life stationary applications. The P-LCA model is developed with Activity-Browser and with a modified version of Ecoinvent 3.7 including background scenarios implemented with PREMISE. The optimization model is a Mixed Integer Linear Programming algorithm developed with Python. The model requires as inputs the environmental burdens of battery manufacturing and recycling per GWh calculated with P-LCA. The minimization constraints are the material flow analysis equations that express the correlations between the parameters as a function of time. The proposed approach determines what batteries shall be manufactured and recycled with temporal resolution and it calculates the optimal management of waste cells and secondary resources. As highlighted by the complexity of the results, these outcomes would have been hardly predicted with an *ex-ante* combination of the parameters of the model as in comparative scenario-based assessments.

#### 5.06.A.T-04 Prospective Optimization of Production Systems in Global Supply Chains

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Whether and to what degree emerging technologies could reduce environmental impacts depends on many choices along their supply chain. Life cycle inventory (LCI) databases depict a detailed process-based representation of products' supply chain with a relatively high technology and geospatial resolution. However, processes in LCI databases are connected in a pre-determined way, representing the current state of production systems. If the goal and scope extend the assessment task by asking for the best production system according to one or multiple objectives, this fixed LCI structure does not allow system-wide optimization via technology choices. Mathematical programming, on the other hand, can efficiently screen through the possible connections across the supply chain in order to determine the best configuration. While the combined use of life cycle assessment (LCA) and optimization has been proposed elsewhere, prior works focused on small systems where LCA data is typically included as cumulative impacts and/or inventory entries for the foreground system. Here, we present a Python-based framework that allows embedding complete LCI databases in optimization problems with the aim of exploiting the high technology and geospatial resolution of these databases, adding also a temporal resolution through the use of future scenarios generated by Integrated Assessment Models (IAM) via the premise package. In contrast to existing works, using the whole technosphere matrix rather than a truncated version also allows for choices at any point across the supply chain (i.e., electricity supplies, means of transportation, raw materials suppliers, etc.) as well as production regions. We demonstrate the capabilities of this framework through the global methanol production system. We find that currently only a handful of regions provide the necessary boundary conditions (e.g., low-carbon intensity of electricity grid and availability of CO<sub>2</sub> as feedstock) to produce environmentally favourable methanol via electrolytic routes. The production of grid-fuelled methanol becomes attractive in energy system decarbonization scenarios, but a decentralized production spread across most regions of the world is required to keep the stress on local electricity grids low.

#### 5.06.P-Th374 Revisiting the Challenges of Ozone Depletion from a Prospective Life Cycle Assessment Perspective

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Several challenges for prospective life cycle assessment (LCA) related to each of the four phases of LCA have been identified by previous reviews. The strategies required to address these challenges are specific to each impact category. Currently, the main focus of prospective LCA research is the assessment of climate change. Still, there is a lack of understanding regarding the specific challenges of other impact categories, for example, the ozone depletion potential (ODP). Therefore, this work presents a review of recent studies regarding current ozone layer trends, future ozone layer modelling and characterization to define strategies for assessing the ODP in prospective LCA studies. In addition, a case study comparing different fertilizing practices is presented to demonstrate how these strategies could affect the results. It was found that the phase-out of ozone-depleting substances (ODS) due to the Montreal Protocol is currently not well represented in the ecoinvent database, resulting in large overestimations of the ODP by banned substances. These overestimations will be more important for prospective studies as the use of banned substances decreases. The review has also shown that, to date, anthropogenic N<sub>2</sub>O emissions, instead of halocarbons, are the most important contributor to ozone depletion. However, the current standard characterization models for ozone depletion have not yet covered these emissions. In addition, several interlinkages with climate change were found. Based on these insights, recommendations are given for future work to improve the quality of inventory modelling and ODP impact assessment in prospective LCA. For example, strategies for N<sub>2</sub>O characterization in prospective LCA will require geographical, temporal and scenario-based differentiation, as the ODP of N<sub>2</sub>O depends on the atmospheric temperature, CO<sub>2</sub>, CH<sub>4</sub> and chlorine levels. More generally, this work showcases the importance of analyzing the challenges of prospective LCA for each impact category individually and collectively, due to potential interlinkages.

#### 5.06.P-Th375 Future Impacts of EV Battery Raw Materials

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Battery electric vehicles are seen as a key technology to decarbonize the transport sector. Electric vehicle (EV) batteries are a key component of EVs and associated with considerable production related environmental impacts. These impacts are likely to considerably evolve in the future due to the energy transition, technological learning and improvements, and other factors. Within the HiQ-LCA project we are developing scenarios for future supply chains of virgin and secondary battery raw materials. The presented research will address, amongst others, the following research questions: How will future environmental impacts related to EV batteries and raw materials evolve? What are the most important drivers for improvements? The research is based on prospective life cycle inventory data generated within the project and uses prospective background LCI data to consider the energy transition and other important developments. We show that EV batteries and raw materials will have substantially reduced environmental impacts in the future, albeit not for all impact categories and not in all scenarios.

#### **5.06.P-Th376 Approaches and Challenges of Assessing Future Environmental Impacts Associated with Metal Supply: A Systematic Review**

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Metals have an important role in the global economy. With the energy transition, the demand for many metals is expected to sharply increase in the future. Although many studies apply prospective LCA to assess future environmental impacts of metal supply, the methods have not yet converged to a common approach. This study aims to provide an overview of these studies and their approaches, following 2 research questions: 1. Which metals have been addressed by previous prospective LCA studies and what are their expected future supply impacts according to the identified studies? 2. What are the studied parameters of the metal supply chains, the applied scenario modelling approaches, and data sources used?

We performed a systematic literature review to identify studies which assess future environmental impacts due to the supply of metals. This includes publications about absolute impacts of global metal demand, but also relative impacts assessed by comparative LCAs of emerging technologies. For these studies, we analysed both the results and the methods to integrate prospective elements in the LCA models focussing on the choice of parameters, background scenarios, data sources and modelling approaches.

The literature review yielded 40 papers. We found that the majority of publications investigate bulk metals like Cu, Fe and Al. Most studies investigate relative impacts (i.e. per kg metal produced). Fewer studies also address absolute impacts of the total future demand; however, these mostly agree that absolute environmental impacts associated with global metal demand are likely to increase. Moreover, the results show that the majority of studies assess CO<sub>2</sub> emissions, while other impacts are less often investigated.

Furthermore, we found that the parameters considered most frequently are future ore grades, recycling shares, and energy efficiency. Background scenarios were primarily energy scenarios, which were most often electricity scenarios from the integrated assessment model IMAGE. Background scenarios modelling other developments are less common. Overall, the review reveals a wide variety of parameter choices, scenario modelling approaches and data sources.

This study stresses the necessity to reduce environmental impacts of metal supply. Moreover, it highlights the need for guidelines for prospective LCA as well as for the documentation of modelling choices, LCI and scenario data to facilitate transparency and sharing of LCA scenarios in the community.

#### **5.06.B Prospective Life Cycle Assessment of Emerging Technologies**

##### **5.06.B.T-01 Combining Ex-ante Life Cycle Assessment with Scenario-Discovery and Conditional Probabilities to Assist Decision-making under Distinct Degrees of Incertitude**

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In times of ecological emergency, prioritizing investments and allocation of scarce time and resources to specific technological directions is crucial. Ideally, prioritizing should be done before initiating uncertain exploration of novel directions. For instance, should we invest resources in looking for new microalgal compounds to enhance fish health in European aquaculture? Ex-ante LCA can assist such policy-making. However, providing estimates of environmental impacts at the very beginning of a technological exploration which is inherently chaotic comes with substantial degrees of incertitude.

While the ex-ante LCA community often addresses uncertainty in its models, theoretical advances within Post-Normal Science have highlighted the need to differentiate the different degrees of incertitude applying to models and data. In this context, “uncertainty” is a specific degree of incertitude characterized by the incapacity to propose reasonable probabilities for events. If the knowledge about the probabilities is sufficient, the incertitude belongs instead to the domain of risk. More than a semantic debate, the presentation of uncertainty as risk, as often done in ex-ante LCA, entails caveats and overconfidence in the decision-making process.

A way of dealing with parameters for which no distribution can be proposed is to assign values to them within what-if scenarios. A conditional probability of impact can therefore be expressed within these scenarios, as a projection of knowledge conditional to the realization of specific values for uncertain parameters. However, generating scenarios for early-stage developments is challenging and the risk of overlooking important potential configurations is high. Scenario-discovery is an alternative in which algorithms discover scenarios of interest for the decision makers by exploring the output space of a model. The decision makers can eventually focus on estimating the likelihood of the discovered scenarios only.

In our work, we combine ex-ante LCA with the distinction of degrees of Incertitude, conditional probability, and scenario-discovery in a case with substantial incertitude. We demonstrate the approach on a case in which policy-makers should decide whether resources should be invested in looking for new microalgal compounds to enhance fish health in European aquaculture. In this work, we build a bridge between LCA and Post-normal Science concepts and methodologies to provide sound decision-making assistance.

#### **5.06.B.T-02 A Procedure for Prospective Life Cycle Assessment in Materials Development – The Case of Carbon Fibre Composites**

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Life cycle assessment is a powerful tool for quantifying the environmental impacts of products and services and is an essential part of product development. It can, for example, be used to identify hotspots to find ways to keep environmental impacts as small as possible. Assessing a system under development requires a prospective approach. A problem in prospective life cycle assessment (pLCA) is, however, the lack of data, which can lead to inconclusive results. The prospective context, however, also implies that there is still room for process changes and improvements. This paper will present a work procedure for pLCA that was grown out of a multi-year project aimed at developing lignin-based carbon fibres for composites. The intention with this contribution is to provide practitioners guidance on how LCAs in early stages of material development can be handled.

When starting the project, there were no data available for the production of the fibres, still efforts needed to be made to identify hotspots in the life cycle of carbon fibre composites and to show the possible influence of transitioning to a lignin-based fibre, as well as to identify other important routes for decreasing the environmental impacts. The meta-analysis found that a shift to lignin and recycling could decrease the environmental impact of carbon fibre composites, but that this is heavily dependent on the allocation approach used.

To further explore the influence of the allocation approaches, two studies were performed: One for assessing the influence of allocation approaches to lignin production and one for assessing allocation approaches for recycling of composites. As the intention was to apply the allocation approaches in a pLCA, both studies included an assessment on how sensitive the approaches were to changes in, for example, prices. The outcome was that many allocation approaches are sensitive to the temporal settings of the study, in particular regarding demand and quality of the fibres.

When different technology routes and allocation approaches had been identified, the findings were applied in a case study of carbon fibre composites in road vehicles. The end-result, where carbon fibre composites reduced the vehicles' environmental impact in almost all futures, should be seen as an indication of the possible future environmental impacts of the system under study and can provide guidance to technology development.

#### **5.06.B.T-03 Transforming Residual Biomass into Food and Feed – Conditions For an Environmental Success**

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This study aims to assess the environmental relevance of “waste-to-nutrition” strategies, that is, strategies transforming residual biomass streams into edible ingredients. Albeit increasingly promoted, these approaches rely on emerging technologies, as showed by the lack of scaled primary data and the heterogeneity of reported lab/pilot-scale datasets. In turn, their future performances are strongly related to uncertain technological efficiency improvements. Therefore, the implications of deploying waste-to-nutrition pathways are poorly understood, and it remains uncertain to which extent these can generate environmental benefits compared to current residual biomass management practices.

We propose to fill this gap by introducing a comprehensive life cycle assessment (LCA) model paired with an advanced global sensitivity analysis (GSA) procedure enabling not only the cross-comparison of 27 different biomass management strategies (15 emerging waste-to-nutrition pathways and 12 conventional management pathways), but also to unravel the necessary conditions required to ensure their environmental success.

To this end, feedstock- and process-dependent parametric inventories were elaborated for each pathway, allowing to explore wide sets of technological performances and biomass compositions. Assumptions and modeling granularity were harmonized, and 293 independent technological parameters were documented. Paired simulations were performed simultaneously with the Morris method over the 27 biomass management pathways, screening the whole range of forecasted technological performances. Besides yielding common GSA results (e.g. sensitivity ratios), this strategy also provides the means to assess effects of individual (or sets of) key parameters on pathways ranking through statistical analysis. The possibilities offered by this LCA model are illustrated on a case study focusing on the french context, where the management of eleven representative local residual biomass streams is assessed. All simulated results were already obtained, but the analysis is still ongoing (conclusions to be delivered at the conference).

On top of proposing the first integrated environmental assessment of waste-to-nutrition strategies, this study also contributes to expand the prospective LCA toolbox by illustrating how the Morris method and statistical analysis can be combined to support decision-making on resource allocation or bioprocesses ecodesign.

#### **5.06.B.T-04 Prospective LCA of Brown-seaweed-based Bioplastic: From Pilot To Industrial Scale**

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Seaweed is gaining momentum as a bioresource for different applications, such as food and feed processing, medical applications

and bioplastics Seaweed-based bioplastic is considered an emerging technology from an LCA perspective. It is important to assess early-stage technological development to identify environmental hotspots. However, the data available for these emerging technologies is generally only available at a lab or pilot scale (TRL 4-7). Upscaling methods are needed to develop a life cycle inventory (LCI) for the prospective LCA at industrial scale. We use the consequential LCA approach to assess the impacts of increasing the demand for this novel bioplastic. In this work we upscale experimental lab- and pilot-scale data to 4000T/year. The model under assessment includes seaweed cultivation, seaweed biorefinery, film fabrication and end-of-life modelled as composting of the biodegradable film. We exemplify how various upscaling techniques are combined and utilized at various life cycle stages. The methods consist of a combination of qualitative and quantitative data to prepare for the upscaling of seaweed farming and the process simulations for biorefineries and film production. Namely, interviews for the seaweed cultivation and process simulations for the biorefinery and film fabrication. Seaweed experts were interviewed to anticipate the upscaling of seaweed cultivation and its limitations. The mass and energy flows resulting from the process technology simulation are used in the LCI. An uncertainty analysis will also be performed given the intrinsic uncertainties in emerging technologies. Regarding qualitative results, seaweed cultivation is expected to grow in the future. However, there are different limitations to consider when upscaling seaweed cultivation, such as the carrying capacity of ecosystems, the seaweed species, and the available technology. The preliminary results show a carbon footprint of 2,6 kg CO<sub>2</sub> eq. at pilot scale. Although pilot-scale results might not represent the real impacts, they provide a glance of the potential impacts at the industrial scale.

#### **5.06.B.T-05 The Environmental Sustainability of New Ways to Produce Benzene, Toluene, and Xylene**

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The petrochemical industry is one of the world's largest industries and significantly contributes to greenhouse gas (GHG) emissions globally. Producing petrochemicals from renewable feedstock instead of fossil fuels may reduce these GHG emissions and lessen wider environmental impacts. For the aromatic petrochemicals benzene, toluene and xylene (BTX), several production routes have recently emerged that use alternative feedstocks, including biomass and plastic waste. However, a thorough understanding of the environmental impacts of BTX production via these routes, and how they compare against each other and against conventional, naphtha-based BTX production is missing. Here, we assess the environmental impacts of bio-based and mixed plastic waste-based BTX production at a commercial scale in 2022 and at industrial scale in 2050, as compared to conventional BTX production. We employ both a prospective LCA approach, as well as an absolute sustainability assessment using the planetary boundary concept (PB-LCIA) to provide recommendations towards more sustainable BTX production. This study shows the future potential to reduce GHG emissions deploying BTX production based on waste and renewable feedstock in combination with electrification of the processes and a renewable electricity mix, leading to GHG emission reductions up to almost 60%. However, even though the alternative BTX pathways reduce impacts, it does not necessarily mean they are environmentally friendly alternatives, as our research shows that impacts do not stay within the planetary boundaries. To further reduce the climate change impact of mixed plastic waste-based BTX production the release of its fossil-based carbon content should be limited. For bio-BTX production, lower impacts in biomass cultivation related impact categories are necessary to avoid trade-offs and could be achieved by switching to other biomass such as lignocellulosic waste streams. Nonetheless, this study implies that a future petrochemical industry producing aromatics from recycled and bio-based feedstock can lower environmental impacts to a large extent as compared to conventional, fossil-based feedstock.

#### **5.06.P Prospective Life Cycle Assessment of Emerging Technologies**

##### **5.06.P-Th377 Choosing the Functional Unit of an Emerging Technology: The Case of Digital Tools in Agriculture**

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There are strong expectations around the mobilisation of digital tools to support the transition to agricultural systems based on agroecology to reduce environmental impacts of food sector. Thus, digital tools are gradually being introduced into agriculture whether for data collection, data analysis or to support field action. As emerging technologies, there is a need in assessing the environmental impact of their use in the agricultural context. To date, little has been done.

Prospective Life Cycle Assessment (PLCA) is a way to assess environmental impacts of emerging technologies, which can have unknown consequences or use at the time they are designed. As a consequence, PLCA raises methodological issues regarding the different steps of LCA framework (goal and scope, inventory, impact assessment and interpretation). This work focusses on the goal and scope and more specifically on the choice of the functional unit (FU). The current practice in agri-food sector is to deal with FU related to mass of food or surface of cultivated areas. We carried a literature review on environmental impacts of digital tools in agriculture. None of the authors discussed the relevance of mass or area based FU for these systems, with no thoughts on the suitability between the function of the tool and the FU. This work aims at objectifying the choice of the FU given an agricultural digital tool.

We identified in the literature several classifications of agricultural digital tools, given their place in the data supply chain or the technology. However, none of these classifications were based on the function of the tools, which can make challenging the choice of the appropriate FU. We built a functional classification of agricultural digital tools and proposed associated relevant FU.

Functions were grouped into four sub-functions (time savings, variable rate application, money savings and ethic), each of them being linked to one or two FUs except for ethical sub-function. Indeed, this last sub-function remains difficult to quantify. There might also be missing functions given the huge amount of digital tools and their emergence.

This work designs a decision tree to frame and standardise the choice of the functional unit when applying PLCA to agricultural digital tools. It helps improving quality and comparability of LCA publications. More generally, this work is a first step towards a guideline for LCA applied to agricultural digital tools.

#### **5.06.P-Th378 Early-Stage Decision-Support Tool for the Environmental Analysis of Chemicals Applied to Ethylene Synthesis**

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In CO<sub>2</sub>-based chemical process development, Life Cycle Assessment (LCA) studies are desired as early as possible to verify targeted global warming impact reductions and to avoid environmental burden-shifting. LCA studies are effortful and require expertise to ensure meaningful results, especially in early-stage LCA, when available data on processes is limited. With a shortage of LCA practitioners and plenty of chemical processes in development, companies need a way to quickly and effectively perform LCA of chemicals to provide environmental guidance.

We therefore present ESTIMATE (Early STAGE Impact Assessment Tool), a publicly accessible Excel tool that enables chemists and chemical engineers to perform methodically robust early-stage LCA of CO<sub>2</sub>-based chemicals.

For three of the most common goals in process development, we standardize LCA-methodologic assumptions and, thus, minimize the prior knowledge required from the user. The minimum required user input for data generation is the stoichiometric equation, from which ESTIMATE calculates the Life Cycle Inventory (LCI), scaled to the functional unit. The LCI can be adapted as data availability increases. To support data generation, ESTIMATE provides estimation methods recommended in the literature to close data gaps for the LCA of chemicals. ESTIMATE then calculates environmental impacts in multiple impact assessment methods while considering different decarbonization scenarios for the most relevant process inputs, such as reactants and utilities. Finally, the tool output includes figures showing contribution analyses, scenario analyses, and an overview of results in all impact categories according to the selected impact assessment methods.

We apply ESTIMATE for the example of CO<sub>2</sub>-based methanol production with subsequent ethylene production in a methanol-to-olefins (MTO) process. In particular, we focus on how the ESTIMATE tool can contribute to process development at different levels of data availability and, in this way, lower the threshold for applying LCA in early development stages.

#### **5.06.P-Th379 Prospective Life Cycle Assessment of a Smart Battery Cell**

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The spatially resolved acoustic, mechanical, and ultrasonic sensing for smart batteries (SPARTACUS) project developed a prototype of a smart battery cell that uses sensors to detect degradation and failure mechanisms during the traction battery operation. This study presents the impacts on climate change (CC) of the SPARTACUS prototype compared to a state-of-the-art (SoA) battery cell based on a prospective life cycle assessment (LCA).

The system boundaries were cradle-to-gate, and the functional unit was 1 kWh of delivered energy. The prototype assembles 3 polyimide (PI) layers (PI#2, PI#3, and PI#4) on a commercial 12 Ah pouch cell. On PI#2 and PI#3, acoustic sensors are used to localize material changes. PI#2 also contains temperature sensors. On PI#4, rubber compression sensors detect mechanical stress and pressure distribution to monitor deformation. A cell management system (CMS) and a pre-processing (PP) board were also included.

The life cycle inventory considered primary data obtained on a lab scale. Two SoA battery cells were cycled for the use stage under the Worldwide Harmonised Light Vehicles Test Procedure (WLTP) cycles. The prototype is assumed to have a 20% higher cycle life. The life cycle impact assessment was conducted using IPCC GWP100a characterization factors. A superstructure prospective database was used. The prospective LCA assessed climate change impacts in 2030, with 2020 as the reference year. The storylines for the assessment were the SSP2-base and SSP2-RCP 2.6 (2°C climate target).

The CC impact of the prototype is at least 160% bigger than the SoA battery cell in all scenarios. The smart cell impact on CC was reduced from 2020 to 2030 in all scenarios. In all years and scenarios, the greatest contributor to CC originated in PI#4 (23% of the total contribution), followed by PI#2 and PI#3 (both accounting for 14% of the impacts). In 2030, in the more stringent scenario (RCP 2.6), the CC impacts of the prototype were reduced by 21%.

Despite its improved performance, the results showed greater impacts on CC for the prototype than the SoA battery cell.

However, its impacts are likely to decrease in future scenarios. The next steps to reduce the impacts in the prototype manufacturing phase are identifying the necessary sensors, identifying the marginal impacts of each sensor layer on the use phase performance and assessing cell recyclability.

#### **5.06.P-Th380 Prospective Life Cycle Assessment of Hemp Fiber Production Versus Conventional Glass Fiber Production**

**Hanie Zarafshani, Ponnapat Watjanatepin and Karel Van Acker, KU Leuven, Belgium**

Although bioeconomy-related innovations may play a significant role in contributing to the support of a more circular and decarbonized economy, it is crucial to ensure that these technologies are bringing more benefits than burdens at an early stage of development. In addition, identifying and minimizing the trade-offs is essential to direct investment and technological developments toward a sustainable economy.

Assessing the environmental impacts of technologies and products at an early stage is a challenge and it is also true for biobased products.

Here, prospective life cycle assessment plays a significant role. Prospective LCA has become more prominent in the evaluation of the environmental impacts of developing technologies, although there are still some methodological issues with the integration of scale and time. To overcome the mentioned challenges, the scenario analysis was investigated as one significant method of performing the PLCA to address some of these challenges.

Scenario analysis looks at the future environmental impacts of technologies and their products. It assesses how technologies and their byproducts will affect the environment in the future. In addition, scenario analysis will be utilized to evaluate a variety of variables that affect the evolution of such processes.

In this study, the environmental performance of hemp fibre production was compared to glass fibre production at TRL 6 or 7 through an explorative scenario approach for the various pathways in the future (2030 and 2050) in Europe.

In this project, a combination of a causal loop diagram with an LCA flow chart aims at linking, in a visually explicit way, the technical parameters, comprehensive-picture surrounding parameters, and the LCI model itself.

Because the deployment of bio-based product technologies is affected by a variety of technological and socio-economic aspects, the creation of future scenarios in a transparent, consistent, and trustworthy manner has proven challenging.

To tackle these issues, a scenario analysis using the PESTEL method was carried out, taking into account numerous variables that affect the development of bio-based product processes, such as biomass availability, climate conditions, biomass feedstock availability, increase in technological performance or substitution of virgin wood for biomass feedstock.

Finally, the LCA results were calculated for the developed scenarios based on the future's background database.

#### **5.06.P-Th381 Reinforced Polymers for Rotor Blades, With or Without Carbon Fiber? A Comparison Through Life Cycle Assessment**

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Germany expects to become climate neutral, and scenarios foresee an expansion of offshore wind energy (OWE). Despite diverse pathways, scenarios describe a significant growth of OWE's cumulative installed capacity. Both Prognos CN 65 and CN 60 depict continuous growth over the years, whereas the Kopernikus basis scenario allocates OWE expansion after 2030. In addition, the nominal capacity of offshore wind turbines (OWTs) has risen considerably since their market launch, with learning rates between 8 and 19 %. Scenarios describe different cumulative capacity trajectories, in turn, different improvement rates over the years, for example, weight reduction paces (because processes become more efficient) or more effort in R&D. Vestas and Gamesa promise to produce OWT up to 15 MW but have opted remarkably different technologies. As for the rotors, Vestas made them of reinforced polymers (RP), and has increased rotor diameter by 5 % without weight gain by combining carbon fibre and glass fibre (CFRP/GFRP). Siemens made its rotors mainly of GFRP. While carbon fiber enables longer and more durable rotor blades, the material has a high environmental impact, e.g., 70 kg CO<sub>2-eq</sub>/kg in comparison to GFRP (3 kg CO<sub>2-eq</sub>/kg). Ultimately, small quantities of carbon fiber could have a detrimental effect on the environment, especially if no measures are taken to improve its performance. This study performs a scenario based life cycle assessment (LCA), compares 2 equivalent rotors (made of GFRP and GFRP/CFRP) and evaluates their impacts to climate change in 2030 and 2050. The study utilizes futurized version of ecoinvent 3.7.1 derived from Premise, combining improvement in both background and foreground systems. The study considers improvement over the years based on cumulative capacity trajectories given by German scenarios, escalates the weight into a 15 MW OWT (rotor 222 m), assumes both rotors – GFRP and GFRP/CFRP – weigh equally and calculates the amount of carbon fibre based on the strength-to-weight ratio of the materials. The annual electricity generation is 82 ± 8 GWh (GFRP rotors) and 84 ± 8 GWh (GFRP/CFRP rotors). Under the same conditions (i.e., location, hub height, distance, and operation speed), the study estimates that 5 % larger rotor diameter means 2 % more GWh per year. However, GFRP/CFRP rotors represent 60 % more emissions to climate change than GFRP rotors in 2030. Despite significant improvement in 2050, the difference is nearly 30 % more emissions.

#### **5.06.P-Th382 Assessing a Novel Multifunctional Autonomous Vehicle Using Electricity Consumption as Functional Unit**

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Inner-city mobility requires large amounts of land use. This is caused by parking and a large number of vehicles per capita.

Another issue with inner-city mobility is a lot of traffic jams causing inefficient fuel use. Autonomous electric vehicles are able to resolve these challenges. By using a car-sharing system the amount of vehicles would drastically decrease which also frees up parking space. Using an autonomous system to connect each vehicle should reduce traffic jams due to smart braking.

Furthermore, these vehicles will not only be used for person- and goods-transport but a plethora of functions, such as street cleaning and public park maintenance. The challenge in this case is the functional unit of this multifunctional system. For normal transport a functional unit can be '1 km of driving'. However, some functions do not require any mobility and are not included in this functional unit. Therefore, a functional unit has to be implemented which incorporates an interface between all functionalities. Because this multifunctional system is completely electric all of the functions require a certain amount of electricity. Consequently, to tackle the challenge of functional unit a change of perspective is proposed. Instead of looking at production or delivering of a certain function the functional unit focuses on the consumption, in this case the consumption of 1 kWh of electricity. The hypothetical functional unit will be 'the consumption of 1 kWh of electricity from the local grid to perform one of the possible functions'. This functional unit is expected to encompass all functionalities and normalize their impacts through a common denominator, the total amount of kWh consumed. Changing the perspective of the functional unit

towards a consumption point of view enables the interfacing of different functionalities of a product. This facilitates the assessment of other multifunctional products with Life Cycle Assessment.

#### 5.06.P-Th383 How Will Large-Scale Manufacturing of MXenes Impact the Environment?

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MXenes are two-dimensional advanced materials that have become an important focus of nanomaterials research since they were first created in 2011. MXenes can have a wide range of compositions – their formula is  $M_{n+1}X_n$ , where M is a transition metal (e.g., titanium or vanadium) and X is either carbon or nitrogen. The most widely studied MXene is  $Ti_3C_2$ , which has a high electric conductivity that enables a variety of potential applications, for instance as electrode material in batteries. However,  $Ti_3C_2$  production is still restricted to laboratory syntheses, mainly by etching with hydrofluoric acid (HF). To date, no environmental assessment of its potential future production has been performed, neither at laboratory scale nor large scale. In this study, a prospective life cycle assessment (LCA) of large-scale manufacturing of  $Ti_3C_2$  is performed in order to fill this knowledge gap. The goal of the study is to assess different  $Ti_3C_2$  synthesis routes and pinpoint their environmental impacts with a particular emphasis on climate change, energy use, and mineral resource scarcity. This analysis also identifies the manufacturing process steps that have the largest impacts and provides recommendations on how impacts of large-scale production of  $Ti_3C_2$  can be reduced. The LCA is a cradle-to-gate study with a functional unit of 1 kg of  $Ti_3C_2$ . Two routes for producing  $Ti_3C_2$  are considered: the HF etching and the in-situ HF synthesis. The distinction between these two routes is that the first adds HF as an etching chemical, whereas the second does not add HF but instead forms it through reactions involving other chemicals. Data on synthesis procedures, material inputs, and energy requirements are obtained from state-of-the-art synthesis descriptions of  $Ti_3C_2$  MXenes in the scientific literature as verified by technology experts in the field. These procedures are then scaled up using process calculations. The sensitivity to different parameters is tested in terms of future scenarios, including different future electricity mixes. Preliminary findings indicate that impacts are dominated by the production of the titanium powder, which constitutes the titanium input to the  $Ti_3C_2$  production. This implies the need to investigate less impacting production routes for titanium to considerably reduce impacts of  $Ti_3C_2$  MXenes.

#### 5.06.P-Th384 Prospective Comparative Life Cycle Assessment of Ethylene Production Through Photoelectrochemistry Vs. Steam Cracking

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We present the lessons learned from applying LCA in the project LICROX (<https://licrox.eu/>), aiming to develop a low-TRL prototype for a photoelectrochemical cell (PEC) for converting sunlight, water and  $CO_2$  into organic molecules, with special focus on ethylene. As part of the project, an LCA study was outsourced. The study was a cradle-to-gate, consequential LCA, where PEC-ethylene is compared to ethylene from steam cracking. As part of the LCA, a consequential model for steam cracking was developed. Execution of the LCA was done in 2 iterations: a 1<sup>st</sup> iteration to identify hotspots, and a 2<sup>nd</sup> iteration to refine the model. The study had to be put on hold for 1 year, to give the consortium more time to have a clearer definition of their new technology. Data collection and the life cycle inventory for the PEC technology was extremely challenging, due to a lack of data by the consortium on many aspects that an LCA needs to address. The consortium provided data on some of the PEC components, on the PEC manufacturing process and on the operation of a theoretical plant. A huge effort in gap filling was needed, including using data for a similar technology, using basic stoichiometry for the production process, literature on production of many different types of basic materials, as well as expert judgement. Interpretation of results was mainly focused on GHG emissions. The results for steam cracking showed a relatively low footprint (0.2 kg  $CO_2e/kg$  ethylene), as a result of adopting a consequential approach. The footprint of LICROX ethylene is higher (5.4 kg  $CO_2e/kg$ ), due to consumption of chemicals during plant operation, as well as due to the impact of infrastructure. An improvement analysis was performed to show the effects of different choices in the design of a hypothetical LICROX plant, or simply to test more optimistic assumptions. Four independent scenarios were tested, as well as a fifth one combining the previous four. The results of this improvement analysis were used to identify potential design choices as well as process variables that matter the most to improve this technology. The study has shown the importance of incorporating LCA expertise in project consortia, rather than have it outsourced. The LCA proved useful to the LICROX consortium to make them reflect on many aspects that might not be apparent to the technology designer, but which prove to be important to understand the environmental credentials of an emerging technology.

#### 5.06.P-Th385 Prospective LCA of Four Climate Positive Technologies

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Most scenarios targeting to limit an increase in global temperatures to 1.5°C above pre-industrial levels, include in their proposed pathways a mix of solutions among which the removal of greenhouse gases from the atmosphere. This project targets developing and maturing promising technologies that remove and permanently store  $CO_2$  and other greenhouse gases from the atmosphere thus generating negative emissions. The four climate-positive solutions investigated in this project are: direct capture of  $CO_2$  from seawater, direct capture  $CO_2$  from air, conversion of biomass into hydrogen and /or heat, and methane removal from air. For all four pathways the last step will be the permanent storage offshore in the Norwegian continental shelf. For the biomass conversion, there are two pathways investigated: farming of algae and biomass from waste flows.

To determine their true potential for net negative emissions, a framework with system boundary which includes all upstream and downstream emissions is essential. Thus, we will apply the “cradle-to-grave approach” from life-cycle assessment methodology to each of these different technologies to evaluate the potential environmental impacts and assess their individual potential for net



negative emissions. In addition, since these are emerging technologies, the LCA shall help to identify the most critical parameters for further technological optimization in addition to highlighting potential alternatives for the choice of processes, energy sources, optimal location of sites, materials as well as end of life treatments. Each of these technologies has a different value chain with distinct processes, but we aim to develop a framework which enable fair assessment and comparison of their performances. Thus, we will investigate the use of a common functional unit for all these climate-positive technologies (1 ton of CO<sub>2</sub>-eq. removed and stored). In addition, we will address the issue of upscaling these technologies and thus we will qualitatively investigate the requirements and potential impacts.

#### **5.06.P-Th386 Ready-made Composite LCA Inventory Development For High-volume Production of a Drone Frame**

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The design and manufacture of the next generation of delivery drones is complex and material choice is multifaceted due to the different characteristics of the available alternatives. Carbon fibre composites are the most suited material to fabricate drone frames due to its mechanical properties and low weight. Carbon fibre can be combined with thermoplastic or thermoset resins to create a composite. The key advantage to manufacturing composites using thermosetting resins is the low viscosity required to impregnate the fibre preforms. The disadvantages of thermoset resins include a limited shelf life, longer cure times, among others. On the other hand, the challenges with thermoplastic resins are that they are solid at room temperature and require high temperatures and pressures to consolidate the composite due to their high viscosity in molten state.

The most common methodology to assess the environmental impacts of a drone airframe production is the Life Cycle Assessment (LCA). To create an inventory for the assessment data is collected from the materials and processes involved in the manufacturing of the composite part. A large volume production requires manufactures to rely on ready-made composite sheets from other producers. This circumstance adds a layer of uncertainty into the assessment as little information about composite sheet production is disclosed by the producers besides what can be found in datasheets. The production of pre-impregnated sheets (prepregs) using thermoplastics as matrix and carbon fibre as reinforcement has been modelled using data available in the literature and adaptations from the Ecoinvent database. The same was done to the production of organo sheets (Pre-consolidated reinforced thermoplastic laminates - RTL) and thermoset prepreg.

This work will present the methodology developed to bypass the lack of available information regarding carbon fibre composite sheets for high volume production to enable a comparative LCA study between thermoplastics and thermoset alternatives. The result has shed light into the absence of data to assess large scale composite parts production based on the current available base material (prepreg and RTL). Moreover, information about the data available in the database has also been tricky to work with as some of the process for polymers are described as “plastic” transformation with little information about its operational parameters.

#### **5.06.P-Th387 Comparative LCA Study for Assessing the Potential Environmental Benefit of a Photocatalytic in Relation to a Conventional Paint**

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The Life Cycle Analysis (LCA) presented in this study is undertaken in the LIFE VISIONS project and aims to compare the environmental impacts for selected impact categories related to the paint sector, between a conventional paint and the innovative photocatalytic paint with TiO<sub>2</sub> nanoparticles, which was developed in the frame of the project. The photocatalytic paint promotes indoor air quality improvement by degrading NO<sub>x</sub> and VOCs air pollution, as well as reducing energy consumption by lowering ventilation needs. As the scope of the study is a comparative evaluation of the total environmental impact of the two paint products, the system includes both the manufacturing and the application phases. Therefore, the boundaries are set from cradle to grave to include the paint application and end-of-life processes, while incorporating the cradle to factory-gate for taking into account the secondary data related to the manufacturing line. For this purpose, paint production data (composition, energy and water consumption) for both conventional and photocatalytic paint were provided by the Greek paint industry involved in the project and used as primary input data to create the inventory. In addition, regarding the photocatalytic paint, two different databases were applied in combination with a process modelling approach in order to fill the data gaps related to the materials required for the synthesis of the photocatalytic TiO<sub>2</sub> nanoparticles. Particularly, for the application phase, data were additionally collected from real scale case studies. The resulting environmental impacts were calculated for a number of different application conditions for the purpose of investigating the hypothesis of the comparative sustainability advantage of the photocatalytic product. Regarding the results, the process contribution analysis highlighted the use of ammonia solution as the main cause for the higher environmental impact of the photocatalytic compared to the conventional paint in the manufacturing phase. However, this is outweighed by the environmental advantage of the photocatalytic product when including the application phase, due to the energy savings in the rooms/buildings painted.

#### **5.06.P-Th388 Prospective LCA on the Design of New Refractories for a Greener Steelmaking Process**

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Being one of the most energy-intensive industries, steel manufacturing is among the most challenging sectors to decarbonise. This

project aims to describe and quantify possible innovative green steelmaking processes through a comprehensive approach, with a focus on new refractory materials and their impact on the process.

Steel is essential to our society, and its usage has grown dramatically in recent decades, with an increase of annual global production of crude steel from 850 Mt in 2000, to 1869 Mt in 2019. In 2019, the building and infrastructure sector is the first steel user, followed by the mechanical equipment, transport and metal products sectors. Iron and steel industry has a considerable environmental impact, especially related to energy consumption, greenhouse gas emissions, resource depletion and water pollution. Therefore, sustainable production of iron and steel is a huge hard task, essential to preserve our environment.

To face the challenge, this study analyses the impact of new refractory materials on the global environmental burden generated by the steelmaking process. In the first phase, strategies to reduce the impact of new refractory materials are evaluated, by optimizing production routes and raw material sourcing. In the second phase, the usage of these new refractories in steelmaking is investigated, by the quantification of how the change of their properties can impact on the overall environmental footprint.

Moreover, to further improve the sustainability of the process, the EAF (Electric Arc Furnace) has been chosen as the core of the steelmaking, and the recycle of refractories is under discussion. For both the phases, the key interest methodology LCA has been chosen to quantify the global environmental footprint.

The relevance of the study relies in the comprehensive and complex approach to the green steel problem, taking advantage of the well-known tool LCA to promote a joint and prospective vision of the product over its entire lifecycle, to address both to the refractory producers and the steel industry. Moreover, the direct collaboration with the two production sectors will allow to obtain a big quantity of real data for the inventory creation. The result of the study should give a powerful tool to renovate steel-making processes, by providing alternative production routes that are not so far from the reality and that could be easily implemented, helping to reach the “green targets” in the short and medium term.

#### **5.06.P-Th389 Comparability of Emerging Technologies at Different Stages of Development Regarding LCIA Scores Using the Example of a New Recycling Technology for PET-Containing Waste**

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The ongoing climate change forces the chemical industry for drastic steps to reduce its CO<sub>2</sub> emissions. Circular Economy, especially the recycling of plastics is an import part for reaching this goal, but not all plastic waste can be recycled today and new recycling technologies needs to be developed. In order to legitimate the substitution of conventional, highly optimized and established technologies with new technologies which are not yet as well known, their ecological benefit needs to be proven. This makes it necessary to accompany the development of new technologies with a consistent and transparent ecological assessment in order to meet the requirements of an alternative and sustainable circular economy for plastics. Those prospective LCAs aims to assess the impact of emerging technologies in industrial scale and addresses the problem of the different technology readiness levels (TRL) of the processes routes which needs to be compared.

A detailed prospective cradle-to-gate assessment is carried out for the newly developed recycling process revolPET. revolPET is a Back-to-Monomer recycling (BMR) process for recovering terephthalic acid (TA) and ethylene glycol (EG) from PET containing waste streams, e.g., polyester textiles or opaque or multi-layer packaging. As this is an emerging technology, the current state of development is not direct comparable to the established fossil-based production routes of the monomers. For this reasons, different methods are used for data acquisition, intending representativeness of the future industrial scale and scenarios are presented for some possible technical solutions of not yet implemented process tasks. The influence of the technology readiness level as well as possible process optimization methods are quantified and discussed.

For a better understanding of the uncertainty caused by the state of development, the foreground flows are classified according to the data sources and the state of implementation of the individual unit operations. This quantitative assessment of the result leads to a more accurate comparability with alternative production or recycling routes, taking into account the stage of development and data availability.

#### **5.06.P-Th390 LCA of Phosphorus Recovery from Dairy Wastewater for Fertilisers**

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Phosphorus (P) is a finite, non-renewable mineral resource that is used primarily in fertiliser production. The scarcity and the increasing demand for this finite material led the European Commission to include this resource in the critical raw material list in 2014. Therefore, considering its importance and its limited availability, an alternative to using mineral phosphate for fertiliser production is required. The interest in the development of technologies with regard to nutrient recovery from organic waste streams has increased in recent years, and a potential input waste stream for these technologies is dairy wastewater (DWW). This contribution reports on the use of life cycle assessment (LCA) to analyse the life cycle environmental impacts of different scenarios for P products recovery from DWW, and the use of the P products as fertilisers in agriculture. The assessment of these emerging technologies was done with prospective LCA, studying the technologies in early stages of development but modelling them as more developed in the future. The assessed scenarios include combinations of different DWW treatments, conventional and innovative, emerging P recovery processes, and agricultural activities. The recovery processes use liquid by-products obtained from the wastewater or sludge treatment processes to produce products rich in P. The preliminary results suggest that the addition of P recovery technologies to the DWW treatment with required modifications will not increase the global warming potential or cumulative energy demand much and that those technologies recovering P from the liquid phase will result in lower impacts than those that recover from sludge.

## **5.06.P-Th391 The Relevance of Early-stage Carbon Footprinting in Sustainable Product Design: The Case of Paper-based Printed Electronics**

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Novel printed electronics are projected to grow and be manufactured in the future in large volumes. In many applications, printed electronics are envisaged as sustainable alternatives to conventional (printed circuit board-based) electronics. One such application is in the semi-quantitative drug detection and point-of-care device called 'GREENSENSE' that uses paper-based printed electronics. This assessment analyses the carbon footprint of GREENSENSE to identify and suggest means of mitigating disproportionately high environmental impacts, labelled 'sustainability hotspots', from materials and processes used during the pilot- and lab-scale production which would be relevant in high-volume applications.

Firstly, a life cycle model traces the flow of raw materials (such as paper, cellulose nanocrystals, and nanosilver) through the three 'umbrella' processes (circuit printing, component mounting, and biofunctionalization) manufacturing different electronic components (the substrate, conductive inks, energy sources, display, etc.) that are further assembled into GREENSENSE. Based on the life cycle model, life cycle inventories are modelled that map out the network of material and energy flow throughout the production of GREENSENSE. Finally, from the environmental impact and sustainability hotspot analysis, both crystalline nanocellulose and nanosilver were found to create material hotspots and they should be replaced in favor of lower-impact materials. Process hotspots are created by manual, lab-, and pilot-scale processes with unoptimized material consumption, energy use, and waste generation; automated and industrial-scale manufacturing can mitigate such process hotspots. This assessment illustrates how prospective environmental impact assessment of novel technologies can serve as an early warning tool for material scientists and product developers, thereby informing sustainable product design.

## **5.06.V Prospective Life Cycle Assessment of Emerging Technologies**

### **5.06.V-01 Comparative Evaluation of DACCS Technologies Considering Location and Energy Sources Using LIME3**

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The AR6WG3 stated that the introduction of Negative Emission Technologies (NETs), which directly remove CO<sub>2</sub> from the atmosphere, is essential to achieve a carbon-neutral society by 2050. Research studies on NETs show that in addition to the benefits of carbon removal, there are also disadvantages to ecosystems and capital goods. This study analyzes the environmental impact of carbon removal, land use change, resource consumption, and water consumption of two technologies, Direct Air Carbon Capture and Storage (DACCS) and Bioenergy Carbon Capture and Storage (BECCS), which are expected to be implemented after 2050 for reasons of CO<sub>2</sub> removal and external cost. The results of the analysis of environmental impacts and the calculation of external costs associated with these environmental impacts will be presented in this study. For DACCS, the analysis is based on multiple scenarios for two technologies, solvent-based and adsorbent-based, considering electricity and heat sources for each location. In the calculations, a meta-analysis was performed for DACCS and BECCS to collect background data; for DACCS, the analysis was based on existing literature data collected. Secondary data was utilized due to the difficulty of collecting primary data. Multiple scenarios were then simulated and evaluated. For BECCS, the Palm Kernel Shell in Malaysia derived land modification impacts were assumed for power generation. The value conversion of environmental impacts was performed using the integration factor in LIME3, an LCIA method developed by Itsubo et al. This method quantifies the importance of protecting each damage in monetary units by analyzing the additional tax expenditures required to reduce them when human or ecological damage occurs. The results of the analysis showed that in BECCS, the external cost per ton carbon removed was 15 to 200 times higher than in DACCS, with land alteration from plantations as the major factor. The DACCS was a major factor in the external costs derived from land use change impact by implementation of photovoltaics. For future studies and research, DACCS should be further evaluated in detail and expressed in terms of environmental advantages/disadvantages of the target technologies in the form of value conversion, so that these technologies in the carbon removal price study.

### **5.06.V-02 Multiple Functional Units' Selection in Prospective LCA for Better Representation of Emerging Technologies in Materials Science: A Case Study of Supercapacitors**

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Conducting a life cycle assessment (LCA) on emerging technologies of complex multi-functional nature presents novel challenges in the functional unit selection process. In opposition to incumbent technologies with a determined application that benefit from a straightforward selection of functional units, allowing for seamless comparison between product systems. Therefore, prospective LCAs should adopt multiple functional units whenever possible to improve their comprehensiveness, transparency, and utility. Thus, offering a viable extension of the conventional functional units (e.g., mass, pieces) that may not clearly represent technologies' actual application. In this manner, various application scenarios are explored predicting the technology performance under different conditions, which allows technology developers to take an informative decision. Supercapacitors (SCs) act as an ideal case study, given their intermediary location on the energy density versus power density chart. SCs exhibit competitive performance with mainstream and established devices such as capacitors and batteries in terms of both power and energy density. Moreover, the measurement of these two properties involves another parameter which is discharge current density, changing the discharge current density impacts the value of energy and power density substantially. As the discharge current density value emulates working conditions such as the application's energy requirement, it is also worthy to consider it in the life cycle impact assessment results.

Benchmarking three SCs enabled by either aqueous electrolytes or an ionic liquid based on power and energy density at 1 and 2 A/g discharge current density resulted in interesting conclusions. Aqueous electrolytes powered SCs emitted up to 60 times more

CO<sub>2</sub>-eq compared to SCs powered by an ionic liquid, this trend was more prominent when increasing the current density to 2 A/g. On the other hand, ionic liquid enabled SCs had 2-10 times lower environmental impacts across all categories when compared based on power density at 1 A/g. In contrast, increasing the current density to 2 A/g narrows the gap between these supercapacitors to the point where an aqueous electrolyte based SC became environmentally advantageous in two midpoint indicators, namely terrestrial acidification and terrestrial ecotoxicity. This case study proves that functional unit selection can substantially impact LCIA results and prospective LCAs conclusions.

#### **5.06.V-03 Combined Technico-Economic Analysis and Life Cycle Assessment of Microalgae Production as Alternative Feedstuff**

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Agriculture and particularly animal husbandry exert environmental pressures. Feed concentrates are one driver of the environmental impacts of meat production, calling for more environmentally friendly alternatives. In this context, microalgae are gaining attention. While studies on microalgae as alternative biofuel exist in literature, evaluating their economic and environmental sustainability as alternative feedstuff brings along a new set of questions. What is the realistic application of the product? What is the reference system to compare the product to? How should the future state of the technology be represented and can its future economic profitability be evaluated? These questions will be addressed first by implementing a combined technico-economic analysis (TEA) and life cycle assessment (LCA). The system under study includes the microalgae production in the bioreactor and the bioprocessing to the final feed. An LCA model will be programed in Python using Brightway2 and lca\_algebraic packages based on literature and from laboratory-specific information. The inventory flows expected to change with time will be parameterized to ease the environmental assessment of a potential technology scale-up. Each parameter will be assigned a laboratory-scale value, ideally taken from measurements, an industrial-scale value, relying on scale-up relationships and expert judgments, and a probability distribution function (pdf) to represent the range of these future states. The TEA model will rely on simple process-based relationships coupled with economic values. Here too, parameters will describe the economic flows and their values correspond to the three levels mentioned. Monte Carlo simulations relying on the pdfs will provide ranges of future environmental and economic impacts. Microalgae is often presented as a substitute for the soya bean part of the animal diet, implying that it has similar nutritional properties. Soya bean is a major supply source of the amino acid lysin, has a high protein content, and shows a good digestibility. Evaluating in a second step whether microalgae fulfill or surpass all these requirements and how they can be embedded in animal diets, will help to define the bioprocessing step and the reference system. This research will discuss methodological questions around the comparability of future and current feedstuff and provide insights into the environmental and economic sustainability of microalgae as alternative feedstuff.

#### **5.07 Reducing Plastics Impacts: Integrating Risk Assessment, Life Cycle Analysis and Material Flow Analysis Towards a Circular Economy**

##### **5.07.P-Mo363 Modelling the EU Plastic Flows Towards a Circular Plastic Value Chain: Approaches, Data Sources and Hotspots**

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As understood by the European Union (EU) Plastics Strategy, plastics is currently managed in way that leads to severe environmental impacts limiting a “circular” plastic value chain. Several other EU policies actions have been put forward to address the plastic challenge, striving for a circular plastic value chain and dealing with the benefits of an improved plastic recycling system. Material Flow Analysis (MFA) is commonly employed in literature to model plastic flows. MFA are commonly developed adopting a top-down approach or a bottom-up approach, with different results due to the underpinning assumptions and data limitations. This study aimed at establishing a top-down MFA model for the whole value chain of plastics in the EU27 for the year 2019, focusing on 9 sectors and 10 polymers. The assessment shed light on the main data gaps and inconsistencies concerning available data and it provided estimates for less explored sectors, such as the textiles and clothing, healthcare, and fishing. Estimates indicate that 4.46Mt of plastic recyclates are produced and consumed in the EU27 territory. On average, the EU27 recycling rate was equal to 19%. Total plastic losses amounted to 4% of the total plastic production, mostly occurring in the use phase. Scenarios for the year 2025 were prepared by following expected trends in the plastic value chain and industry targets (e.g., the EU target of 8.8Mt of recycled plastic consumed in EU27 by 2025). Only when multiple actions are combined (i.e., improved waste collection, interventions on mismanaged waste, etc.) the 8.8Mt target could be achieved, with results ranging from 9.11Mt to 11.13Mt of recyclates consumed by EU plastic converters. Results underline that to fulfil the EU ambitions and industry targets, significant efforts are needed to further improve the granularity and details of plastic flows in the EU. Such enhancements could include better sector-specific and polymer-specific data for less explored sectors, coupled with in-depth knowledge of recyclates’ fate, and both losses and mismanaged waste flows. Considering these key commitments for actions at the EU level, an improvement and rethinking of the plastic value chain is mandatory and should be driven by an up-to-date knowledge of all its many hotspots.

##### **5.07.P-Mo364 The United States Federal Plan to Address Microfiber Pollution**

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Due to growing concerns over the presence and persistence of microfiber pollution in the environment as well as their potential

ecological and human health impacts, the United States Congress mandated that the Interagency Marine Debris Coordinating Committee (IMDCC) develop a Report on Microfiber Pollution. Under Section 132 of the Save Our Seas 2.0 Act of 2020 (P.L. 116-224), the Report on Microfiber Pollution must include: (1) a definition of microfiber; (2) an assessment of the sources, prevalence, and causes of microfiber pollution; (3) a recommendation for a standardized methodology to measure and estimate the prevalence of microfiber pollution; (4) recommendations for reducing microfiber pollution; and (5) a plan for how Federal agencies, in partnership with other stakeholders, can lead on opportunities to reduce microfiber pollution during the 5-year period beginning on such date of enactment. This Report on Microfiber Pollution provides an overview of this issue and efforts completed to date, and the Federal Plan further outlines priority actions to be implemented over the next five years. Through a series of federal agency workshops, a questionnaire, and feedback from experts in the field (in the form of an Expert Advisory Committee composed of representatives from relevant academic, government, and industry sectors), a Federal Plan was drafted to begin mitigating microfiber pollution. This five-year plan focuses on addressing microfiber pollution from production stages (textile manufacturing and production), through use stages (everyday use, fiber mitigation opportunities via filtration devices), and disposal stages. Public feedback on the Report on Microfiber Pollution was solicited during a 30-day public comment period allowing anyone to provide feedback on the report and draft Federal Plan. The Federal Plan will prioritize research needs to further understand the impacts associated with microfibers, engage cross-governmental agencies and cross-sector stakeholders to address this issue, and evaluate solutions to address microfiber pollution from textiles and non-textile sources alike. This presentation will discuss a multi-pronged approach to research, prevent, and drive solutions and mitigation measures for one prominent type of marine debris, microfiber pollution.

#### **5.07.P-Mo368 Self-reinforced Polylactic Acid (SR-PLA) is More Resistant to Releasing Microplastic than Polypropylene (PP) after UV Irradiance**

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The over-dependence on petroleum-based polymers has led to a series of environmental issues, including the occurrence and persistence of microplastic (MP), in the global ocean. Polymers made from a natural-sourced feedstock, known as bio-based polymers, are hypothesized to be more sustainable alternatives. However, our knowledge remains limited about their degradation and fate in the marine environment. Plastic debris in the marine environment is known to break down to smaller sizes and release MPs under ultraviolet (UV) radiation. A number of studies have provided evidence of the release of MP from larger debris under UV radiation in laboratory conditions, observed via weight loss, changes in average particle size and SEM observations. However, the most direct and quantitative evidence of MP formation, i.e. observation, identification and enumeration of MPs formed after UV radiation, is limited. Until now, only a few studies have assessed the weathering of bio-based polymers and their capacity to form MPs. Here, we aim to assess the MP formation of a bio-based polymer, self-reinforced polylactic acid (SR-PLA), and a petroleum-based polymer, polypropylene (PP), during weathering by UV radiation. To do so, we exposed 3D printed cylinders (surface area=4.7 cm<sup>2</sup>) of SR-PLA and PP, immersed in filtered natural seawater, to accelerated UV radiation for 57 and 76 days, simulating 18 and 24 months of mean natural solar irradiance in Europe. Dark controls (i.e. sealed vials from UV, n=6) were incubated in the same conditions for the same duration. To identify, characterise and quantify the formed MPs, we used a combination of fluorescent microscopy, infrared technology ( $\mu$ FT-IR) and image analysis. The average concentration of released SR-PLA MPs ( $\geq 50\mu\text{m}$ ) per surface area was  $3.9\pm 2.0\text{ \#}/\text{cm}^2$  in UV exposures and  $1.6\pm 0.8\text{ \#}/\text{cm}^2$  in dark controls. For PP, this was  $53.4\pm 46.3\text{ \#}/\text{cm}^2$  and  $0.9\pm 0.9\text{ \#}/\text{cm}^2$ , respectively. For both polymers, higher MP concentrations were found after 76 day UV radiation ( $p<0.05$ , Dunnett's test) compared to samples kept in dark. The PP cylinders released significantly more MPs than SR-PLA after UV exposure ( $p<0.05$ , Dunnett's test), indicating that the bio-based polymer SR-PLA is more resistant to releasing MPs than the petroleum-based polymer PP after UV irradiance. We anticipate that our results will contribute to assessing the sustainability of future bio-based polymers and to informing a transition process to more sustainable plastic materials.

#### **5.07.T-01 Mass Flow Analyses and Emissions of Microplastic and Bisphenols From Waste Electronical and Electric Equipment Plastic (WEEEP) in Norway**

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Waste of electrical and electronic equipment (WEEE) is one of the fastest growing waste streams in the EU. Approximately 20% of WEEE is plastic (WEEEP). Increased recycling of WEEEP is beneficial to reduce virgin plastic production and environmental emissions of microplastics (MPs) from landfills. In Norway, WEEEP is mainly sent to material recycling, however, certain fractions are challenging to handle and recycle as their toxic components pose a substantial threat to the environmental and human health. Plastic containing brominated flame retardants (BFRs) is mainly incinerated. However, recent reports show that increasing amounts are being recycled in Norway, indicating that regrettable recycling of BFRs in plastic may be occurring. Cables are one of most plastic rich WEEE items, often containing up to 50 % polyvinylchloride (PVC), as well as high concentrations of toxic plasticizers like bisphenols to prolong flexibility and lifespan. In Norway, cable plastic is mainly landfilled despite this being the least preferred option of waste management in a circular economy. WEEEP, especially cable and BFR-plastic, have high concentrations bisphenols. The landfilling of WEEEP, such as cable plastic, may thus lead to increased emissions of MPs and

BPs, by dust emissions to atmosphere or leachate to surface and ground water. We present a mass flow analysis (MFA) of BPs and MPs in WEEEP, with focus on BFR- and cable plastic in Norway between 2018 and 2020. Our MFA shows that landfilling of 4 900 tonnes cable plastic resulted in annual accumulation of over 300 kg of BPs, this is predicted to lead to environmental emissions of 50 kg bisphenols and over 5 kg of MPs from landfills. Incineration and thermal destruction efficiently remove 200 kg of bisphenols from the WEEEP MFA, however recycling of 20 000 tonnes of non- BFR- or cable plastic may have contributed to 200 kg bisphenols transferred to plastic recyclates. As such, the present study emphasize a need for improvement of the waste management of WEEEP by reducing landfilling of cable plastic, and tracking the WEEEP recyclates to avoid its use in food packaging or products for vulnerable groups. Some of these issues can be effectively resolved by increased safe and sustainable design of electric and electronic products, through avoiding problematic polymers like PVC and hazardous substances like bisphenols.

#### **5.07.T-02 Towards Including Microplastics in LCA: Effect Factors for Microplastics and Tire Wear Particles for Soil Ecotoxicity**

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Even though the main emphasis on microplastic (MP) pollution is on aquatic environments so far, the MP abundance in soils is expected to be 4 to 23 times higher than in oceans. In addition, emissions to soils were estimated to be 40 times higher than to surface water in Switzerland alone. Even though the number of studies which investigate the effects of MPs on soil organisms is increasing, there is still a crucial gap in quantifying the effects of MPs on soil ecosystems. Thus, it is not yet possible to assess the environmental sustainability of microplastic-producing products, processes, or systems. To fill this gap, the integration of MPs into Life Cycle Assessment (LCA) tools has a crucial importance. For this purpose, the first effect factors of MPs, and tire particles for soil ecotoxicity were derived in this study. A database of soil ecotoxicity studies with microplastics was collected from the scientific literature. Ecotoxicity data regarding plants and invertebrates such as earthworms and springtails were collected to include different relevant ecological levels. This hazard dataset with studies published until June 2022 was used to build probabilistic species sensitivity distributions that uses uncertainty factors to transform acute experimental data to chronic data, and different type of dose descriptors to EC10<sub>eq</sub>. Highest observed no-effect concentration data points were excluded from the MPs database, while they were included in the tire particle database due to the lack of data. Effect factors were then calculated based on the hazardous concentration affecting 20% of the species. Results showed that the hazard data set includes 48 and 18 data points for MPs and tire particles representing 17 and 8 different species, respectively. The mean value of the effect factors for MPs was found to be 0.55 PAF kg/g, while it was much smaller for tire particles with 40.34 PAF kg/g. The effect factors of MPs and tire particles calculated in this study provide the first data towards including MPs and tires into LCA studies for soil toxicity. Full characterization factors to assess the impacts of MP related products, or processes are urgently needed to reveal the effects on soil ecotoxicity, and to support decision-making processes. A further update of the data set should be developed by excluding HONEC values, especially for tire particles once more ecotoxicity values are available in the literature.

#### **5.07.T-03 Life Cycle Inventory and Environmental Fate of Tire-Wear Particle and Textile Washing Microfiber in Japan**

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Full life cycle impacts of plastics (and substitutes) consideration is mandatory to achieve sustainable solutions to end plastic pollution. This requires considerations of fossil-based and biobased feedstocks for plastic production, plastic chemicals, and additives, plastic degradation pathways and intermediates, environmental impacts, ecotoxicological and human health effects, socio-economic costs, etc. Life Cycle Assessment (LCA) is a tool to assess life cycle impacts. However, the present LCA methodological approaches have limitations in including the impacts of microplastics in the LCA. This impact integration into LCA needs both the life cycle inventory (LCI) and life cycle impact assessment methods developments. Tire-wear particles (TWP) and synthetic textile microfiber (TMF) are estimated to be 1.41 and 0.26 million tons/year. The present study estimated TWP and TMF emissions at the prefectural level and 10 km grid level in Japan for six vehicle classes, developed a fate model based on drainage and wastewater treatment systems (WWTPs) in Japan and estimated the fate factors (FF) for several compartments. TWP emissions per unit distance of travel by different vehicle classes have been estimated for Japan. A WWTPs model was developed (ArcGIS) based on the features (treatment level and capacity, location) of the centralized 2114 units and decentralized treatment systems. The TWP and TMF fate in several compartments were estimated using the developed WWTPs model. Estimated TWP emissions for motorcycles and heavy vehicles were 111 to 1664 g/life-of-tire respectively. The total TWP emission for Japan in the year 2019 was 33,796 T/year. The TWP and TMF emission LCIs were obtained for the prefectural and 10 km grid levels (data not given). There are significant differences among the prefectures on the emissions per unit population (and unit GDP). The percentage of combined sewer coverage and WWTPs coverage are the governing parameters for the TWP and TMF FFs to water in Japan respectively. Even though the urbanized prefectures (i.e., Tokyo) emit more TWP the FFs to water become lower (0.23). On the other hand, prefectures like Hokkaido, where the road network is spread over a large area and has less combined sewer coverage, the FFs become higher (0.92). However, in the case of TMF, the FFs in water are correlated with the WWTPs coverage. Further, the FFs of land are dependent on the WWTPs' sludge management practices (data not given).

#### **5.07.T-04 Integrating Ecotoxicological Effects Caused by Additives on Aquatic Species into Life Cycle Impact Assessment**

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Additive substances applied in the plastic industries leach from plastic litter and may lead to exposure to aquatic biota. The ingestion of these substances potentially cause lethal or sub-lethal toxic impacts. Currently, LCA lacks environmental indicators to assess the physical and chemical impacts caused by plastic pollution in the entire plastic value chain. Substantial research is ongoing to improve the plastic pollution impact assessment within life cycle impact assessment. We aim to contribute to this effort and present here the effect factors for six groups of additives composed of different chemical substances. The groups of additives which are derived from the EF are Alkylphenols, Bisphenols, Benzophenones, Brominated flame-retardants, Chlorinated phenols, and Phosphates. Their functions in the plastic material are stabilizers, plasticizers, pesticides, flame-retardants, and biocides. Data on the dose-response relationship for laboratory-derived toxicity were gathered for different additive substances tested in the aquatic individuals. After compilation, the additives were classified into the broader groups of chemical categories. Subsequently, we analysed and processed the dose-response data collected to derive species sensitivity distributions for different individual groups of additives and then derive Effect Factors (EF), using  $EF = 0.5/(HC50_{EC50})$ . Comparing the dose-response relationship, the higher toxic to the aquatic species are presented by Alkylphenols, followed by Brominated flame retardants and Phthalates. The effect factors provide information on chemical stressors related to the plastic additives on aquatic species of at least three trophic levels. This information is an important advancement for the characterisation factor for aquatic ecotoxicity in Life cycle impact assessment. Advances on this topic contributes to the plastic life cycle assessment which can provide information for stakeholders in decision-making processes as well as guide policies for the application of additives aiming to make the plastic value chain more sustainable.

#### **5.07.P Reducing Plastics Impacts: Integrating Risk Assessment, Life Cycle Analysis and Material Flow Analysis Towards a Circular Economy**

##### **5.07.P-Mo365 A Guide for LCA Modelling of Plastics for Safe, Sustainable, and Circular Transition Planning**

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Used in products, packaging, and medical devices, plastics are pervasive in our daily lives. Yet poor design, remiss waste management planning, inadequate recycling capabilities, and negligent usage have led to unsustainable plastic systems, resulting in detrimental environmental and human health impacts. The transition to a circular plastics future is essential for maximizing resource utilization, addressing the plastic accumulation challenges, and maximizing the use and potential of plastics. Today, transition planning and innovative design are supported through Life Cycle Analysis, which assess the impacts of existing systems or forecast the potential impacts of prospective futures.

However, the usefulness and accuracy of Life Cycle Assessments (LCA) are determined by the data and modelling choices used. Recent reviews have found that more than half of the LCAs on plastics in recycling scenarios are incorrectly modelled, as they are failing to transparently account for plastic additives. The impact of LCAs failing to account for additive composition of the plastics, means they miss opportunities to assess the risks of downcycling, degradation, and contamination in circular systems and may be missing human health and environmental impact hotspots. Furthermore, the datasets available for modelling plastics do not account for all the processes needed to accurately model the impacts of plastic production and recycling systems. How then can LCAs inform transitions to circular scenarios or accurately evaluate the impacts of proposed solutions if the baseline is not adequately modelled to create consistent and representative models of plastic systems?

This research aims to address this challenge by proposing a methodological approach for modelling plastic production and recycling systems to better account for the human and environmental impacts for the various processes, flows, and flowables needed for plastic systems. These approaches aide practitioners in modelling variations in the composition of plastics. This methodology proposes modelling approaches for use with major LCA software and databases to create an accurate base for plastic assessments which can then be modified to better represent the variability of plastic compositions. The proposal of this methodology and case studies demonstrates the consistency of the baseline approach and sets flexible framework which can be adjusted based on the chosen modeling methodology.

##### **5.07.P-Mo366 Global Human Health Effects of Plastic Waste Reduction Strategies: A Hybrid Material Flow Analysis and Life Cycle Assessment Framework**

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Global initiatives are attempting to reimagine linear economies of plastics. Human health impacts across the plastic life cycle are a growing concern but quantification of effects remains scarce. This evidence is critical to ensuring policies for plastic waste reduction maximise co-benefits and mitigate any trade-offs for human health. Using a hybrid Material Flow Analysis (MFA) and Life Cycle Assessment (LCA) approach, our study quantitatively estimates and compares the Disability-Adjusted Life Years (DALYs) associated with the entire plastic life cycle under different policy scenarios between 2016-2040. We build on an existing well-acknowledged MFA, the Plastics-to-Ocean (P<sub>2</sub>O) model, that tests the influence of globally implemented waste reduction and circular economy strategies on marine pollution between 2016-2040. P<sub>2</sub>O provides data for the mass of plastics for each scenario, for each year, and for each life cycle stage of the global MFA system. Our study uses this data paired with LCA to estimate global health impacts by (i) generating modular unitary building blocks representing the DALYs (and related midpoint

impacts) associated with 1 million metric tonnes of plastic polymers for each process and (ii) combining building blocks to represent each policy scenario. Using industry reports, we estimate specific polymer shares equivalent to the categories of plastic products described in P2O. We conduct the LCA in Simapro 9.3, with data from Ecoinvent database 3.8 (cut-off) and published literature, using ReCiPe 2016 impact assessment methodology. In Stata 17 software, we combine yearly mass flows for each policy scenario with LCA impact assessment results for each building block. Final analyses compare DALYs between policy scenarios, considering the relative contributions of midpoint impacts. Analyses are ongoing, preliminary results suggest that P2O policy scenarios reducing virgin plastic production and open burning could provide the greatest co-benefits for health, with possible trade-offs associated with mechanical recycling. Climate change, air pollution and toxicity contribute most to health impacts, whilst data and methodologies to assess macro and microplastic pollution health effects are lacking. Our study will provide a critical analysis of health co-benefits and trade-offs of global plastic waste reduction strategies, generating an interdisciplinary framework that can be extended with emerging research across sustainability concerns.

**5.07.P-Mo367 Linking Material Flow Analysis with Plastic Related Impacts: How to Make Progress with What We Know**  
**Valentina Helen Pauna<sup>1</sup>, Simon Alexander Saxegård<sup>2</sup>, Mafalda Silva<sup>1</sup> and Cecilia Askham<sup>1</sup>, (1)NORSUS AS, Medemblik, Norway, (2)Karlstad University, Norway**

The emission of micro- and nanoplastics (MNPs), its diverse pathways and fate in various ecosystems make the MNP problem a compelling topic in numerous research areas. Furthermore, the MNP discipline continues to rapidly evolve and develop. Therefore, the scientific research regarding the environmental impacts of plastic pollution faces challenges with respect to severe knowledge and data gaps because of the need to quickly develop methods for analyzing MNPs from different perspectives. To address data gaps and link differing perspectives, several international and interdisciplinary initiatives have been underway to bring experts in areas such as mass flow analysis (MFA), risk assessment (RA), ecotoxicology, toxicology, and life cycle impact assessment (LCIA) together to establish data guidelines and to harmonize the efforts of data generators. Our current work aims to use tools, like MFA, to extract relevant information from existing MNP data and link these data to LCIA. In addition, we aim to include new and emerging data that has fulfilled the criterion of our data guidelines, such that they can be used to understand MNP impacts associated with specific product commodities. Some specific cases that will be included are geotextiles, mulch film, slow-release fertilizer, and dolly ropes. This approach could allow us to progress in LCIA and inform stakeholders of possible risks, while also leaving room to include more detailed and accurate data as it is made available.

**5.07.P-Mo369 Wash Cycle Design Can Reduce Microplastic Emission from Home Laundry**  
**Shreyas Patankar, Stephanie Wang, Kevin Landrini, Anneka Vanderpas and Charlie Cox, Ocean Wise Conservation Association, Canada**

Home laundry is a major source of microplastic pollution and is endangering ocean health and, potentially, human health. Globally, the rate of microfibers being released to the ocean is estimated to be around 40,000 tonnes per year, and microfibers of textile origin have been found to be pervasive in even the most pristine environments, such as the Arctic Ocean. To address this pressing concern, we investigate whether adjustments to wash conditions can help to reduce microfiber shedding during home laundry. Building on prior research that studied microfiber shedding variation between different textile materials and constructions, we conducted a series of experiments to systematically measure microfiber shed rates during different wash conditions. Over one hundred washes were conducted using loads of polyester jerseys weighing approximately two kilograms, representative of the weight of a typical consumer laundry load. We found that low intensity wash conditions - generally described as 'gentle' cycles - can reduce microfiber shedding by approximately 70%. Further research is needed to determine the specific relationship between laundry washes and the microscopic breaking and release of microfibers that generates shedding - with a view towards balancing laundry wash quality standards and reduction of microfiber shedding. Future research into life cycle analysis is also needed to understand whether reduction in home laundry microfiber shedding results in increased lifetime of consumer textiles, hence resulting in a net long-term reduction of microfiber pollution in the ocean.

**5.07.P-Mo370 Biodegradable Microplastics: How Do Copolymer Blends Fragment and Biodegrade in the Environment?**  
**Patrizia Pfohl<sup>1</sup>, Markus Rückel<sup>1</sup>, Lars Meyer<sup>1</sup>, Glauco Battagliarin<sup>1</sup>, Patrick Bolduan<sup>1</sup>, Thorsten Huffer<sup>2</sup>, Michael Zumstein<sup>2</sup>, Thilo Hofmann<sup>2</sup> and Wendel Wohlleben<sup>1</sup>, (1)BASF SE, Germany, (2)University of Vienna, Austria**

For specific applications, biodegradable polymers can serve as an ecologically friendly alternative to conventional polymers, because the microbial oxidation and hydrolysis in the environment constitute a circular process that transforms polymer carbon into biomass and CO<sub>2</sub>. For several biodegradable polymers this is shown by monitoring the CO<sub>2</sub> evolution and disintegration over biodegradation time. Here we additionally show that fragmentation is part of the biodegradation process and formed secondary fragments are further biodegraded, not leaving persistent microplastics behind. We apply sensitive microplastic-specific methods to test this hypothesis.

Biodegradation of polymers is not homogeneous, even under standardized conditions. Since biodegradable polymers are often blends, the degradation of the components might differ, e.g. due to variations in the chemical structure, crystallinity and microbial colonization. For a specific biodegradable blend (relevant for food packaging) we found that even the product shape (cryomilled particles, shredded foils, coated paper) influenced fragmentation. This blend consisted of polylactic acid (PLA, susceptible to hydrolysis) and aliphatic-aromatic ester copolymers (e.g. PBAT, susceptible to enzymatic cleavage). To gain a mechanistic understanding of copolymer blend biodegradation we studied fragmentation and biodegradation of several biodegradable copolymer blends under industrial composting conditions (ISO 14855) and compared them against the non-blended polymer matrix. With our previously reported efficient and non-destructive particle extraction processes and  $\mu$ -Raman as well as



fluorescence microscopy we were able to monitor fragment number, shape, size, and identity during biodegradation. In addition, we used Soxhlet extraction to assess not only the solid polymer, but also the hydrolysed fraction.

The combination of these methods allows to compare fragmentation mechanisms of different copolymer blends and helps to answer the question, if persistent microplastic is accumulated because of biodegradation of these materials. For the investigated case studies, we found that intermittently, up to 0.0012% of the polymer mass were fragments < 25 µm, but at 90% CO<sub>2</sub>, less than 10<sup>-10</sup>. This means that biodegradation of the polymer blends led first to fragmentation, but the previously formed fragments can further degrade until completely transformed into CO<sub>2</sub> and biomass without accumulation of persistent microplastic.

#### **5.07.P-Mo371 Estimation of Emission and Transfer of Microplastics to Tokyo Bay, Japan by Material Flow Analysis**

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To reduce the discharge of microplastics (MP) into the aquatic environment, it is necessary to properly identify the source. Since it has been pointed out that there were limitations in identifying the sources of MP by observation and measurement, it is considered effective to identify the major source by a material flow analysis approach. The approach is based on reported data of the use or production amount and their estimated emission factor to the environment.

We focused specific sources of MP; i.e. personal care products (PCP), fiber from clothes and tire wearing particle (TWP), for which we could obtain reliable data of use and emission. We estimated potential emission to Tokyo Bay as 10, 38 and 1800 t/year for PCP, fiber and TWP, respectively. These estimated values included uncertainty, so we applied uncertainty analysis using Monte Carlo simulation to reveal range of variation. Also we estimated the amount of transfer to Tokyo Bay assuming deposition and diffusion parameters of the particles.

### **Track 6: Environmental Policy, Risk Management, and Science Communication**

#### **6.01 Advances in Understanding of the Fate and Toxicity of Metals and Metal Mixtures, and its Application in the Regulation of Metals in the Environment**

##### **6.01.P-We340 Nickel in the North: Evaluating Nickel Risks Under Arctic-Specific Exposure Scenarios**

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Nickel (Ni) is an economically and biologically important trace metal, but due to increased demand globally, environmental contamination may arise. The Arctic is not isolated from these impacts, with substantial Ni production in Northern zones leading to contamination of surrounding environments, with global warming increasing accessibility of reserves and thus risk of continued contamination. Many Arctic freshwaters are predicted to have high Ni bioavailability and thus toxic risk due to their abiotic composition (hardness, pH, dissolved organic carbon), while heterogeneity in marine waters may lead to site-specific increases in bioavailability. Freshwater Ni bioavailability and risk under Arctic exposure scenarios will be evaluated with both acute and chronic Ni exposures in the Arctic relevant cladoceran *Daphnia pulex*. This study will evaluate Ni toxicity in waters that mimic Arctic chemical parameters (i.e., hardness, pH), and will evaluate how altered temperature may modify sensitivity. Incorporation of both lethal and sublethal endpoints will allow for assessment of both overt toxicological effects and the mechanism of toxic action (MOA) in *D. pulex* under Arctic exposure scenarios. Alteration of a single hardness ion (magnesium or calcium) to Arctic observed levels reduced 48-hour median lethal concentrations for *D. pulex* neonates from 4.90 mg/L in control waters to 3.76 mg/L and 1.57 mg/L, respectively. Simultaneous modification of multiple parameters (hardness, pH, temperature) will more accurately mimic Arctic waters and is predicted to further increase Ni toxicity compared to single parameter modifications. Nickel risk in Arctic marine waters will be evaluated using the Arctic green sea urchin (*Strongylocentrotus droebachiensis*). *S. droebachiensis* embryos will be assayed for developmental delays, as well as indicators of Ni MOA under Arctic-relevant exposure scenarios. This will allow for comparison with temperate testing which shows *S. droebachiensis* embryos are highly sensitive to Ni contamination, with a 96-hour effect concentration (EC<sub>50</sub>) of 1.38 µg/L for induction of developmental abnormalities. These results taken together will provide insight into the mechanism and extent of Ni toxicity under Arctic-specific exposure scenarios for both fresh and marine waters, furthering the understanding of the potential risks of Ni in these unique ecosystems.

##### **6.01.T-01 Chronic Metal Mixture Toxicity: Quantitative Reappraisal and Identifications of Data Gaps**

**Charlotte Nys<sup>1</sup>, Karel Viaene<sup>2</sup>, Koen Oorts<sup>1</sup>, Marnix Vangheluwe<sup>1</sup>, Violaine Verougstraete<sup>3</sup>, Hugo Waeterschoot<sup>4</sup> and Karel De Schampelaere<sup>5</sup>, (1)ARCHE Consulting, Belgium, (2)ARCHE, (3)Eurometaux, Belgium, (4)Chemicals Management, Eurometaux, Belgium, (5)Ghent University - GhEnToxLab, Belgium**

In the Chemicals Strategy for Sustainability, the EU Commission calls to systematically integrate the issue of combined exposure into chemical risk assessments. Hence, there is a need to provide scientific evidence on metal mixture effects as input to the discussions on how to tackle the issue of exposure to unintentional mixtures of naturally occurring substances, like metals and inorganics. The current research builds further on a recently published meta-analysis of mixture effects of Cd, Cu, Ni, Pb and Zn to aquatic organisms at the species-level, but expands the quantitative reappraisal to a broader set of metals.

The quantitative reappraisal aimed to answer the following 3 questions: Q1: Which mixture reference model, either Concentration Addition (CA) or Independent Action (IA), is most accurate for predicting chronic metal mixture toxicity at the species level; Q2: How frequently do significant deviations from the mixture reference models, CA and IA, occur; Q3: How accurate is CA for predicting mixture effects at low effect concentrations? To answer these questions, a literature study was performed to identify relevant recent studies (>2007) and the identified (mixture) concentration response data were analysed in a systematic manner. In total, 30 chronic metal mixture studies were identified, covering 24 species and 14 metals in 33 combinations. It was shown that CA results in more conservative predictions compared to IA. However, metal mixture toxicity to invertebrates is most accurately predicted by IA, while there are no clear differences between the models for algae. Most experiments showed non-interactive effects or antagonistic effects, while significant synergisms were less frequently observed, certainly when evaluated relative to CA. CA overestimated mixture toxicity at low effect levels (i.e., 10% mixture effect) by 1.3-fold (median value). The present study largely confirmed the findings from the earlier study that considered fewer metals. Within the current study the following main data gaps were identified: no high-quality data on vertebrates or on mixtures with anionic metals were identified. In addition, more complex mixtures combining 4 or more metals have also been rarely tested. The results of the current study can contribute to the scientific discussions on the implementation of combined exposure into environmental risk assessment (e.g. by possibly refining the ) and will also help to prioritize further experimental efforts.

#### **6.01.T-02 Using Bioavailability to Characterise Risks in European Freshwaters; The Example of Copper**

**Iain A Wilson<sup>1</sup>, Adam Peters<sup>1</sup>, Graham Merrington<sup>1</sup> and Stijn Baken<sup>2</sup>,** (1)*wca environment Ltd., United Kingdom*, (2)*European Copper Institute, Belgium*

Copper is as a naturally occurring essential trace element, that has been regulated in numerous jurisdictions through the use of concentration thresholds. The understanding of the behaviour, fate and ecotoxicity of copper has evolved in the last 20 years, with the concept of bioavailability being now broadly accepted. Despite this, assessments on the exposures of copper have continued to be performed based solely on the dissolved copper concentration. A dataset covering over 17,000 sites in Europe was collated, including bioavailability correction parameters pH, DOC and dissolved Ca. The dataset was used to assess the risk of copper in European freshwaters, with or without consideration of bioavailability. The data was assessed using a variety of compliance metrics used by agencies in Europe, and the conclusions were compared.

When bioavailability is not accounted for, it appears that there is a potential risk to freshwaters across the European continent. This is based on the number of countries with greater than 5 % of sites exhibiting exceedances; the spatial, temporal and extent of exceedances (STE score), and the 95<sup>th</sup> percentile of the calculated risk quotients (RQ). In contrast, when the assessment is performed on the bioavailable concentration of copper none of the three examined metrics indicates a widespread risk.

This assessment, therefore, shows that if bioavailable concentrations are not utilised in a risk assessment the extent, and magnitude, of the risk of copper to freshwaters in Europe can be significantly overestimated. This overestimation may lead to incorrect decisions being made with respect to copper, and potentially other metals, and lead to policies being applied that are not proportional to the risk associated with the substance. It was noted during the assessment that significantly less sites (and samples) could be assessed accounting for bioavailability (77,617 sites compared to 286,185), with the majority of sites that cannot be further assessed being due to a lack of supporting parameters. Therefore, additional focus should be placed on obtaining the required information to be able to assess the maximum number of sites appropriately following available guidance, prior to any policy making decisions are applied.

#### **6.01.T-03 Development of a Machine Learning Model to Estimate the Biotic Ligand Model-based Predicted No-effect Concentrations for Copper In Freshwater**

**Jiwoong Chung<sup>1</sup>, Geonwoo Yoo<sup>1</sup>, Jinhee Choi<sup>2</sup>, Jae-Seong Jo<sup>1</sup> and Jong-Hyeon Lee<sup>3</sup>,** (1)*EH Research & Consulting, Korea*, (2)*School of Environmental Engineering, University of Seoul, Seoul, Korea*, (3)*Magok-dong, Korea*

The copper biotic ligand model (BLM) has been used for ecological risk assessment by taking into account the bioavailability of copper in freshwater. The copper BLMs requires many water quality data such as pH, major cation, dissolved organic carbon. It is not easy to get all of these variables in the water quality monitoring programs. It is, therefore, needed to develop an optimized estimator of PNEC based on BLM. To develop the optimized PNEC estimation model based on an available monitoring dataset, an initial model that considers all BLM variables, a second model that requires variables excluding alkalinity, and a third model using electrical conductivity as a surrogate of the major cations and alkalinity have been proposed. Furthermore, deep neural network (DNN) models have been used to predict the nonlinear relationships between the PNEC (outcome variable) and the required input variables (explanatory variables). The predictive capacity of DNN models was compared with the results of other existing PNEC estimation tools using a look-up table, multiple linear and multivariate polynomial regression methods. Three DNN models, using different input variables, provided better predictions of the copper PNECs compared with the existing tools for four test datasets, i.e., Korean, United States, Swedish, and Belgian freshwaters. Consequently, it is expected that the copper BLM-based risk assessment can be applied to various monitoring data sets, as the most applicable model among the three DNN models can be selected according to the data availability of the collected monitoring database.

#### **6.01.T-04 Air-Water Cross-Contamination by Metalliferous Atmospheric Particulate Matter, Fish Metal Toxicodynamics and Human Health Risk for Its Consumption**

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Seattleable atmospheric particulate matter (SeAPM) containing a mixture of metals, including metallic nanoparticles has increased in the world causing environmental and biota contamination. The bioaccumulation pattern of metals contained in 1 g L<sup>-1</sup> SeAPM after its dissolution into water was evaluated in Nile tilapia (*Oreochromis niloticus*) for 30 days. The human health risk

for its consumption was assessed using estimated daily intake (EDI). SeAPM was collected surrounding an iron ore processing and steel industrial complex in Vitória city (Espírito Santo, Brazil). Water samples were taken daily for physicochemical analyses and, every 3 days for multielemental analyses. Fish were collected every 3 days for metal bioaccumulation determination in the viscera and fillet (muscle). B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Pb, Hg, Ba, Bi, W, Ti, Zr, Y, La, Nb and Ce were quantified in SeAPM, water and fish viscera and fillet by using ICP-MS. Most metals bioaccumulated preferentially in the viscera, except the Hg and Rb which were accumulated mostly in the fillet. The bioconcentration pattern were similar for  $Fe > Al > Ti > Mn > Pb > V > Zr > La > Ce > Y > Ni > Se > Nb > As > W > Bi$  in whole body and viscera being higher than in muscle throughout exposure. Rb, Cd and Hg increased slowly in whole-body, viscera and fillet, but continuously. B and Cr increased in whole-body and viscera remaining high during the exposure period. Zn, Cu, Sr, Sn, Ag and Ta did not differ from controls. The differences among metal bioaccumulation were probably related to species ability to cope each metal and/or inefficient excretion. The risk associated with the daily intake of tilapia fillets exposed to  $1 \text{ g L}^{-1}$  SeAPM would not pose a health risk for adult consumers but indicated that is not safe for children, due to the concentrations of As, La, Zr, and Hg. It is highly worrying mainly considering the emergent metals from which there were no governmental regulations and possible effects are still unknown.

## 6.01.P Advances in Understanding of the Fate and Toxicity of Metals and Metal Mixtures, and its Application in the Regulation of Metals in the Environment

### 6.01.P-We339 In Vitro Bioaccessibility Round Robin Testing for Arsenic and Lead in Standard Reference Materials and Soil Samples

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In vitro bioaccessibility (IVBA) for arsenic (As) and lead (Pb) in soil can be determined using USEPA Method 1340. This method is validated for As and Pb and includes in vivo/in vitro regression equations that enable the use of bioaccessibility data to predict relative bioavailability of As and Pb to better estimate human health risk associated with soil ingestion. NIST 2711a or 2710a are used as standard reference materials (SRMs) in Method 1340. The suitability of using other SRMs were investigated in this study. Following review of various SRMs, BGS119 and EnviroMAT SS-2 were selected based on their cost, availability and the concentration ranges of As and Pb. Five Canadian laboratories participated in Round Robin I to determine As and Pb IVBA in the two SRMs. For BGS119, IVBA ranged from 10.2% to 17.8% for As, while Pb IVBA varied from 67.4% to 86.7%. For EnviroMAT SS-2, As IVBA varied from 16.5% to 27.6% while Pb IVBA ranged from 65.0% to 87.7%. Intra- and inter-lab variability were acceptable with percent relative standard deviations (%RSDs) < 20%. A method for As and Pb IVBA, that included the use of BGS119 as a SRM was drafted for inclusion in the British Columbia (BC) Environmental Laboratory Manual. Round Robin II was conducted using the draft method to determine As and Pb IVBA in the three SRMs (NIST 2710a, 2711a and BGS119) and 10 field-collected soil samples covering a wide range of As and Pb concentrations. Four BC-based laboratories participated, with each reporting three replicate analyses per sample. Total As and Pb concentrations in the soils reported by three of the labs were comparable, however the concentrations reported by the fourth lab were relatively lower. The differences in concentrations were attributed to the use of the <2 mm particle size sieved fraction for the total metal analysis in soils rather than the <150 µm fraction specified in the IVBA method. The calculated IVBA values for the SRMs were within specified control limits while intra- and inter-lab variability were generally acceptable with %RSDs < 30%. Arsenic IVBA values for the field collected samples varied from 0.1 to 60.4 % while Pb IVBA ranged from 7.0 to 121.6%. The lowest IVBA values were measured in mine impacted soils, while the highest values were associated with smelter impacted soils. The study demonstrated that reproducible and comparable As and Pb IVBA results can be achieved by the participating labs using the BC Environmental Laboratory Manual method.

### 6.01.P-We342 Is Assuming Additivity of Single-Metal Toxicity Thresholds a Conservative Approach to Assessing Risk of Ecotoxicity from Elevated Soil Concentrations of Co, Cu, and Ni at Contaminated Sites?

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Multiple metal-impacted soils are often realistic scenarios for risk assessments, but tools to address these are currently lacking. The objective of this work was to evaluate whether assuming concentration addition (CA) of metal mixture effects was conservative for prospective risk assessment of soils that were elevated mainly in Ni and Cu and somewhat with Co, Pb, or As. Observed whole mixture toxicity for field soils with aged metal mixtures was compared to the expected whole mixture toxicity, assuming additivity of prospective single-metal thresholds ("toxic units") for the mixture components. Bioavailability adjusted single-metal toxicity thresholds expected for those field soils were the median hazard concentration affecting 5% of species (HC5-50) from the predicted no-effect concentration (PNEC) calculator and calculated from the species-specific dose-response multiple linear relationships (MLRs), all from the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (EU REACH) dossiers for metals. Generic single-metal toxicity thresholds were based on Canadian Council of Ministers of the Environment soil quality guidelines (CCME SQGs) for agricultural soils. Observed toxicity thresholds were from the community-based risk assessments conducted for Port Colborne and Sudbury, Ontario, Canada. Mostly, prospective single-metal toxicity thresholds were protective relative to the observed toxicity, although that was species or ecological process dependent. The bioavailability-adjusted single-metal thresholds were less conservative than the CCME SQG method, even though the former is based on site-specific EC10 values, and the latter is based on generic EC25 values. When within-site variability in soil properties was used to calculate the 5th and 95th CI for the HC5 sum of toxic units (ΣTUs), CA was conservative for far

fewer endpoints. In addition, the prospective  $\Sigma$ TUs were more conservative predictions of the observed whole mixture toxicities for Port Colborne soils than for Sudbury soils. The most appropriate balance of accuracy and conservatism for identifying low-level risk of the whole mixtures in these soils appeared to be the bioavailability-adjusted HC5-50, which was applicable to many endpoints and 2 quite different exposure concentration ratios.

#### **6.01.P-We343 Does the Concentration Addition Model Become a More Conservative Predictor of Aquatic Metal Toxicity with Increasing Number of Metals in the Mixture?**

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Risk assessment of chemical mixtures is most conveniently regulated following the concentration addition (CA) model. The MAF (Mixture Assessment Factor) has been proposed as a tool to regulate individual chemicals while accounting for unintentional mixture effects predicted by CA. At low chronic effect concentrations, CA on average tends to overestimate mixture effects at the species level. A more accurate alternative for chronic metal mixture toxicity provides the independent action model (IA). A quantifier to assess the deviations of observed toxicity from toxicity predicted with CA is called the MIF (Mixture Interaction Factor), which indicates synergistic ( $MIF < 1$ ) or antagonistic ( $MIF > 1$ ) interactions, relative to CA, if present. However, most research has been conducted with well-studied metals, such as Cu, Zn, Ni, Cd, and Pb, in equitoxic test designs. Less attention has been paid to other metals and environmentally relevant concentration ratios. This study aims to fill this knowledge gap and will further test the hypothesis that IA is generally a more accurate model than CA for metal mixture toxicity. In addition, it will, based on theoretical and mathematical considerations, test the hypothesis that the MIF increases with an increasing number of metals in the mixture.

Ag, As, Ba, Cd, Cr, Cu, Mn, Ni, Pb and Zn have been identified previously as inorganic substances contributing most to predicted mixture risks. The experimental design consisted of testing single metals, and binary, ternary, quaternary and quinary mixtures combinations of those metals. To cover different trophic levels, the test organisms *Raphidocelis subcapitata* and *Daphnia magna* were chosen. For those species, we estimated that in a mixture experiment, five metals are usually sufficient to explain 90% of the toxicity of the mixture pressure (expressed as the sum of toxic units) of the whole mixture.

All tests followed a ray design at environmentally relevant concentrations and concentration ratios based on monitoring data from the European Environmental Agency (EEA) Waterbase. Additionally, an equitoxic ray design based on EC10 values was also tested. Each test was evaluated according to CA and IA, both models were compared for their ability to accurately predict observed toxicity, and the MIF was quantified. This study will contribute relevant experimental data for a better understanding of the joint toxicity of complex, but environmentally and regulatorily relevant metal mixtures.

#### **6.01.P-We344 Chronic Metal Mixture Toxicity: From Data Gap Analysis to the Development of an Environmentally and Regulatory Relevant Experimental Program**

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In the Chemicals Strategy for Sustainability, the EU Commission calls to systematically integrate the issue of combined exposure into chemical risk assessments. The ‘mixture effects of metals’ project within the Metals Environmental Exposure Data Program (MEED) aims to provide scientific evidence on mixture effects as input to the discussions on how to tackle the issue of exposure to unintentional mixtures for naturally occurring substances. More specifically, it aims to focus on those metals and inorganics that are predicted to contribute most to the overall risks of unintentional mixtures in Europe, i.e. the so-called Inorganic-Priority Contributing Substances (I-PCS). In a previous prioritization exercise using European monitoring data, the following inorganics have been identified as I-PCS: Ag, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, V, and Zn. In addition, in a recent meta-analysis, it was observed that there is overall limited (high-quality) data on metal mixture toxicity to aquatic organisms of following I-PCS in freshwaters: Ag, As, Ba, Co, Cr, Hg, Mn, Se, and V. Moreover, there is only limited data for mixtures combining four or more metals.

We present the development of a targeted testing program that aims to cover these data gaps. An experimental program was developed that considers metal mixture toxicity testing with algae (*Pseudokirchneriella subcapitata*) and invertebrates (*Daphnia magna*) at environmentally regulatory relevant concentrations and mixture combinations. The development of the experimental program followed a step-wise approach focusing on the identification of mixture risks of the I-PCS based on species sensitivity data (EC10) from the REACH registration dossiers. The most relevant mixture combinations were identified using a toxic unit-approach applied on the Waterbase, an European aquatic monitoring database. The followed approach ensured the environmental and regulatory relevancy of the experimental program by selecting the most relevant mixture size, metals, metal combinations and metal concentration (ratios) to be tested. The outcome of the experimental program, that is currently ongoing, will increase the scientific evidence on mixture toxicity of inorganics. As such, it will contribute to the discussions on the implementation of combined exposure into environmental risk assessment.

#### **6.01.P-We345 MEED: Progress of the Multiyear Metals Environmental Exposure Data Collection Program to Anticipate Challenges of the EU Zero Pollution Ambition Policy and the Chemicals Strategy for Sustainability**

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The Chemicals Strategy for Sustainability (CSS) is one of the main pillars of the European Union Zero Pollution Ambition, a

building block of the Green Deal. A major novelty of this policy initiative is the introduction of a Mixture Assessment Factor (MAF) to demonstrate safe use and lack of impact on ecosystems from the cocktail of chemicals exposures encountered in environmental compartments. The MAF contributes to the overall objective of reducing *exposures of chemicals to levels that are no longer expected to be harmful to health and the environment*. Knowing that the Green Deal and its climate objectives will result in significant increases of metal volumes and uses for renewable energy (e.g. wind mills, solar cells and EV-batteries), it is crucial for the metals sector to demonstrate that exposure levels of emitted metals will not be increasing proportionally. In addition, the sector needs to demonstrate that exposure to metal mixtures in the receiving environments will meet the challenge of the Zero Pollution Ambition and the MAF requirements, now and under future emission scenarios.

Therefore, the EU metals sector designed a comprehensive “environmental exposure gathering programme” (MEED), complemented by scientific concepts that are development to anticipate the Zero Pollution Ambition as well as the objectives of the EU biodiversity policy. The program (2022-2024), which consists of 6 interlinked projects, is halfway. Some important milestones that have been reached include: the confirmation of the extent of the impacts of a default MAF for the metals sector, the identification of metals that contribute the most to the combined risks in soil and aquatic environments, an update of today’s regional background levels for metals, a review and reappraisal of existing knowledge on metals mixtures & metals-organics mixtures interactions and the design of a smart testing strategy to fill major gaps in the knowledge of mixture interactions. This poster presents the overall progress and intermediate conclusions of the MEED projects while a detailed assessment of each of the individual milestones are presented in complementary posters.

MEED will deliver a huge amount of data that can be used in REACH registration updates and other EU regulatory programs.

#### **6.01.P-We346 Copper Deficiency in Aquatic Organisms: How Should This be Considered as Part of the PPP Risk Assessment?**

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The EU regulation for authorising plant protection products (Regulation (EC) 1107/2009) uses a tiered risk assessment approach for aquatic organisms. Under this approach, if an active substance fails the lower-tier, more conservative risk assessment, then intermediate or higher tier approaches can be used to demonstrate low risks to aquatic organisms. Higher tier refinements can include the derivation of toxicity endpoints from more realistic test systems, such as mesocosm studies. Assessment factors (AFs) are then applied to those toxicity endpoints to derive a regulatory acceptable concentration (RAC) for use in the risk assessment. There is some flexibility in terms of the AF value applied by regulators to derive the RAC, which can increase or decrease the conservatism of the risk assessment.

Copper is widely used in a variety of industrial applications as well as for plant protection products and it has been documented that Copper is toxic at high concentrations (worst case). However, Copper is also an essential nutrient that is required at low concentrations and if these levels are too low this may cause adverse effects (deficiency). In regulatory risk assessments the focus is usually based on toxicity at these high levels but there is little or no consideration for the impacts of deficiency at lower levels. For example, would the AF assigned by regulators drop the RAC below the required level for normal growth and development and should deficiency be considered by regulators for nutrients such as copper? This issue of essential metals having a U-shaped dose-response curve is mentioned in the EFSA statement on transition metals (EFSA 2021), but not in the context of AF-setting. Based on this, a literature search was conducted to investigate the evidence available for copper deficiency, with particular focus on the critical taxa of aquatic invertebrates and fish. This poster discusses if this could then be used to support use of a reduced assessment factor in the aquatic risk assessment for essential nutrients including copper.

#### **6.01.P-We347 Canadian Federal Water Quality Guidelines for Metals**

**Sushil Dixit**, *Environment and Climate Change Canada, Canada*

The National Guidelines and Standards Office (NGSO) of Environment and Climate Change Canada (ECCC) has recently developed many Canadian federal water quality guidelines (WQGs) for the protection of aquatic life for metals (e.g., aluminum, copper, chromium, iron, lead, manganese and rare earth elements). NGSO uses CCME (Canadian Council of Ministers of the Environment) methods to the extent possible. Because the aquatic toxicity of metals is strongly dependent on physical and chemical characteristics of the exposure water, a careful consideration was given to bioavailability and toxicity of specific metals to aquatic organisms under site-specific conditions. The species sensitivity distribution (SSD), multiple linear regression (MLR) and biotic ligand model (BLM) are among the approaches that we have used for guidelines development and their applications to specific metals will be discussed. The WQGs for aquatic life provide benchmarks for the quality of the ambient environment. Where the WQG is met there is low likelihood of adverse effects to aquatic life. WQGs are regularly used to aid in environmental protection by providing targets for acceptable environmental quality, in evaluating the significance of concentrations of chemical substances currently found in the environment, and as performance measures of the effectiveness of risk management activities.

#### **6.01.P-We348 New Fertiliser Regulation - Are You Prepared?**

**Andrew Brotherhood**, *Agilent Technologies, United Kingdom*

With the new European Union (EU) regulations on fertiliser content (FRP 2019/1009) which came into effect from July 2022, fertiliser manufacturers are potentially faced with the need to stand up to new instrumentation and analytical methods to ensure regulatory compliance. Prior testing and composition requirements almost exclusively covered inorganic fertilisers, but the new fertiliser regulations have been updated to also include the growing organic fertiliser segment. The regulations incorporate additional producers that were not previously subjected to such requirements, so companies should understand the implications of the new regulations, identify instrumentation suitable for associated analyses, and compile methods for speciation and total

elemental analysis testing. A variety of approaches, such as those using ultraviolet-visible (UV-Vis) spectroscopy, microwave plasma-atomic emission spectroscopy (MP-AES), and inductively coupled plasma (ICP) instrumentation, including ICP-optical emission spectroscopy (ICP-OES) and ICP-mass spectrometry (ICP-MS), are among the recommended options for performing robust speciation and elemental analyses. Examples of ICP-based instrumental methods applied to determine total elemental content and arsenic speciation are outlined along with the analytical performance standards attainable with such equipment.

#### **6.01.P-We349 Lead Exposure in Marbled Teal and White-headed Duck: What Is the Situation Two Decades After the Ban on Hunting with Lead Shot in "El Hondo" Natural Reserve (Southeastern Spain)?**

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El Hondo Nature Reserve is comprised of two large reservoirs and their associated wetlands hosting more than 400 species, including marbled teal (*Marmaronetta angustirostris*) and white-headed duck (*Oxyura leucocephala*). The wetland was created in the 1920s to provide water for irrigation. Other relevant human activities include hunting and fishing. Millions of lead shots have been widespread in this wetland. Lead (Pb) poisoning in waterfowl due to ingestion of shot pellets from hunting activities has been well documented worldwide. In 2001, a regulation banning the use of Pb shots for hunting in Ramsar sites was approved. Previous studies found high lead pellet densities in sediments 30 years after the ban of hunting.

Ducks were grouped in "before" or "after" 2001 (date of death). Thirty marbled teals (15/15) and forty white-headed ducks (25/15) from El Hondo were studied. Samples of liver, brain, kidney, muscle and bone were collected for analyses of Pb. Mass body measurement, sex, age and physical condition were registered. The gizzards were explored to detect the presence of ingested Pb shots. Lead levels were analyzed in 0.1 g of dry tissue after an acid digestion. Pb concentrations were measured by ICP-MS. TORT-2 was used as reference material.

In both species, "before" group had mean lead concentrations higher than "after" group, however they only were statistically significant in white-headed duck. In this last species Pb mean concentrations in both groups (before and after) were higher than threshold values for lead lethal exposure. However, in marbled teals, animals dead before 2001 had Pb concentrations in tissues corresponding to subclinical exposure and those dead after 2001 had Pb values corresponding with background lead exposure. Females of white-headed ducks had more Pb shot pellets in gizzards than males. Positive correlation was found between mg of lead in gizzard and lead concentrations in tissues.

In conclusion, Pb concentrations in tissues of marbled teals and white-headed ducks from El Hondo decreased significantly since 2001; however, 22 years after prohibition of Pb ammunition in Spanish wetlands, the impact of lead exposure on health in anatidae remains high, mainly in globally threatened white-headed duck. Other additional measures should be considered to reduce lead exposure in this species.

#### **6.01.P-We350 Development of a Machine Learning Model to Estimate the Biotic Ligand Model-based Predicted No-effect Concentrations for Copper Using Indigenous Species in Korean Freshwater**

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Biotic ligand model (BLM) for transition elements is a quantitative ecotoxicity prediction model that can be used to assess ecological risks taking into account the bioavailability of a transition element such as copper. Since a monitoring database operated by the National Institute of Environmental Research (NIER) of Korea provides only a few BLM input variables, the BLM-based predicted no-effect concentrations (PNECs) calculated using estimated values for missing input variables have uncertainty. In this study, a user-friendly tool was developed that simplifies the many input variables and complex procedure required to derive BLM-based PNECs for copper. The electrical conductivity, easily observed by portable measurement, was used as an input variable to surrogate major cations and alkalinity. A deep neural network (DNN) model was used to predict the nonlinear relationship between input variables and PNECs. As a result, copper BLM-based PNECs derived from the sensitivity distributions of 19 native aquatic organisms proposed in the previous study were accurately predicted to within a factor of two. Furthermore, the temporal variation of copper PNECs estimated at detailed monitoring stations in the monitoring database of NIER from 2017 to 2021 were investigated. The copper DNN model requiring only pH, electrical conductivity and total organic carbon can contribute to the application of risk assessment reflecting the bioavailability of copper in various monitoring databases.

#### **6.02 Bird and Mammal Risk Assessment: Now and Preparation for the Future**

##### **6.02.P-We351 The Appropriateness of Using the Rabbit (*Oryctolagus cuniculus*) in the Risk Assessment of Plant Protection Products for Wild Mammals**

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The new EFSA draft Guidance Document on the Risk Assessment for Birds and Mammals proposes to assess the risk of plant protection products (PPP) to generic model species (GMS) for many crop scenarios at tier 1. One of these GSMs, defined as

*medium herbivorous mammal* – and with same body weight and diet composition as the European rabbit (*Oryctolagus cuniculus*) – has a high rate of tier 1 risk assessment failure.

To assess the potential for chronic risk to wild mammals from PPP, two types of information are needed: a) the relevant wild mammal toxicity endpoint and b) the relevant wild mammal exposure scenario. Under the current and the draft new guidance documents, the European rabbit (*Oryctolagus cuniculus*) is used for both: a) as a laboratory test system to derive potential toxicity endpoints for all wild mammals, using the only available laboratory rabbit study (prenatal developmental toxicity) and b) as the relevant potential focal species to assess the risk to medium herbivorous mammals at higher tier risk assessment refinement.

In this platform, we reflect on whether (or when) the use of the rabbit is still appropriate for a realistic, relevant, and robust wild mammal risk assessment in the context of PPP.

We will present and discuss the peculiarities and limitations of the rabbit prenatal developmental toxicity study when used for wild mammal risk assessment, after conducting a novel analysis of the relevance of the maternal and fetal observations, and their potential contribution to the (un)successful recruitment into wild mammal reproductive population.

We will also assess the relevance of the rabbit as the focal species at higher tier risk assessment refinement, after conducting a thorough literature review on rabbits focusing on: European distribution, biology and population dynamics, feeding and habitat preferences, impact of agriculture on rabbits, impacts of rabbits on agroecosystems and rabbit population management in European farmland.

The platform will expand on the methods used and the conclusions available so far and will be supported by relevant posters where further details will be presented.

#### **6.02.P-We352 Effect modelling for Higher Tier Bird Risk Assessment – A Case Study**

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The Guidance Document on the risk assessment for birds and mammals as published by EFSA in 2009 is currently being updated. The new draft guidance document lays out the prospect of using toxicokinetic-toxicodynamic (TKTD) effect modelling for higher tier assessments but also highlights that currently no case studies on the application of those models for the bird risk assessment are available. Here, we present a case study where effect models have been applied as a higher tier option for the bird risk assessment of the fungicide fluopyram.

Reproduction studies with Bobwhite quail and fluopyram, following the OECD guideline 206, revealed that offspring weight is significantly affected by the dietary exposure of the adult quails, which provides the most sensitive endpoint for the bird risk assessment of the compound.

Dynamic energy budget (DEB) models and a physiologically-based toxicokinetic (PBK) model were developed to address this endpoint in a higher tier risk assessment approach. The effect models should adequately represent the process or mechanism leading to the effect. We, thus, tested two different hypotheses for mechanisms that may lead to reduced offspring weight upon the fluopyram exposure of the hen, i.e., the energy hypothesis and the exposure hypothesis. The two hypotheses required different sets of effect models which were tested for their ability to adequately describe the effects observed in the reproduction studies.

Also, a novel egg injection toxicity study was carried out to explore potential effects of chemical residues in the egg.

Our results indicate that the reduction of offspring weight upon exposure of adult quail to fluopyram is likely an effect caused by egg residues rather than a direct (energetic) effect posed on the hen. The model validation with independent data revealed that the combined DEB-TKTD and PBK modelling adequately predicts the observed effects in the reproduction study without any further vertebrate testing. The combined effect model was successfully calibrated and validated and can thus be used for the higher tier risk assessment. The overall modelling approach also offers the potential to replace bird reproduction studies in cases where egg residues and offspring effects are likely to drive the risk assessment.

#### **6.02.P-We353 Integrating Emerging Science to Improve Assessments of Chemical Risk to Wildlife, Challenges and Workshop Recommendations**

**Nico van den Brink<sup>1,2</sup>, John E. Elliott<sup>3</sup>, Elizabeth Power<sup>4</sup>, Clare Lauren Kilgour<sup>4</sup> and Mark S. Johnson<sup>5</sup>, (1)Division of Toxicology, Wageningen University & Research, Netherlands, (2)Dept of Toxicology, Wageningen University & Research, Netherlands, (3)Environment and Climate Change Canada, Canada, (4)Azimuth Consulting Group Inc., Canada, (5)U.S. Army Public Health Center**

In several jurisdictions, Environmental Risk Assessment (ERA) of chemicals includes evaluating the potential risks of chemicals for terrestrial wildlife (mammals, bird, reptiles and some amphibians). In such assessments, the potential for adverse effects from exposures to anthropogenic chemicals at contaminated sites, for new pesticides, and for chemicals in production and/or proposed for wide-scale use is evaluated and assessed. Although ERA has included wildlife-related assessment for decades, the tools and methods to evaluate wildlife related risks have not markedly changed, despite the advancement of new scientific insights and tools. In 2019, the Wildlife Toxicology World Interest Group of SETAC initiated a virtual workshop, based on a special session at the annual SETAC North America meeting in Toronto, Canada. This meeting was organised on the prospect to improve risk assessments for wildlife and facilitate better implementation in chemical regulations. The workshop focused on regulations in the USA, Canada, and Europe that incorporated risks to wildlife species. Following that meeting, four work groups focussed on different aspects of the ERA for wildlife, i.e. problem formulation (WG1), exposure assessment (WG2), effects assessment (WG3), and risk characterization (WG4). In this presentation, we provide an overview of the workshop and results, focussed on the regulations that drive risk assessment. Key focus areas for further development will be presented that may help to improve the quality of predicting of risks of chemicals to wildlife, including recommendations for future research and decision making.

#### 6.02.T-01 Use of the Time Weighted Average Factor in Pesticide Risk Assessment for Birds and Mammals

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The use of time-weighted average (TWA) concentrations for exposure estimations in risk assessment of pesticides has been a topic of discussion and debate. In recent decades, it has been recognized that although many chronic toxicities are likely a result of extended exposure situations, there situations wherein it cannot be assumed the toxicity observed will be the same for different exposure patterns with the same AUC exposure level. That is to say, it cannot be determined by most standard toxicity studies whether the observed toxicity is indeed a result of a certain level of long-term exposure, rather than, for example, a short or peak exposure at a critical time-point or stage and dedicated toxicological test designs are needed to determine whether these may be the case. The Working Group on the Revision of the EFSA Bird and Mammal Guidance document took this into account during the revision of the birds and mammals risk assessment guidance.

#### 6.02.T-02 Focussing on What Matters: A Suggestion for Addressing Vulnerability in Bird Focal Species Selection in Higher Tier Risk Assessments

**Steven Kragten<sup>1</sup>**, Arnd Weyers<sup>2</sup>, Dennis Sprenger<sup>3</sup>, Benedikt Gießing<sup>4</sup>, Raquel Sainz Elipe<sup>4</sup> and Christian Wolf<sup>4</sup>, (1)Syngenta Agro, Germany, (2)Bayer AG - Crop Science Division, (3)Corteva Agriscience, Germany, (4)Tier3 Solutions, Germany

In the registration process for plant protection products (PPP), a higher-tier risk assessment is triggered if no acceptable risk is indicated at the first tier. One higher tier refinement option is the use of a focal species (FS). The fundamental concept is that a risk assessment for the selected FS should protect other species of the same feeding guild as represented by the FS.

The draft revision of the Guidance Document for Birds and Mammals Risk Assessment proposes an approach for FS selection, under which feeding guild, body weight and diet composition are considered more important than frequency of occurrence in study fields (FO<sub>Field</sub>). In addition, it is noted that “*A vulnerable species which is infrequently observed but nevertheless clearly present may be considered a more appropriate focal species for the risk assessment...*”. This implies that the most vulnerable species within a feeding guild in the crop would be a suitable FS. Using the example of birds, we here ask the question: what indicates the vulnerability of a bird species occurring in the relevant crop?

Weyers et al. suggested to aid in FS selection, calculating a species-specific “daily dietary dose” or DDD, which provides an indication of the relevance of a bird species for higher tier risk assessment. The approach by Weyers et al. did not provide criteria to identify a vulnerable species as proposed by EFSA. Here, we present a refinement to the approach by Weyers et al. that helps evaluating if a species is vulnerable, based on real data from FS studies, which can be used as a surrogate for PT in theoretical DDD calculations for the purpose of ranking FS according to ‘vulnerability’.

The example data used demonstrates that FO<sub>Survey</sub> values indicate that three of eight species might make frequent use of this crop for foraging. This is then confirmed by the obtained PT data. The FO<sub>Survey</sub> data are of course not a direct equivalent of PT as the highest FO<sub>Survey</sub> does not necessarily translate into the highest PT. Nevertheless, this example demonstrates that FO<sub>Survey</sub> may be a suitable parameter for estimating the magnitude of the differences in utilisation of a crop among the most relevant species in the absence of PT data.

This approach can possibly be used under the new guidance document for birds and mammals risk assessment and can contribute to a more harmonized approach between Member States on this topic.

#### 6.02.T-03 PT Studies: Practical Implications of the Draft Updated Guidance Document on ‘Risk Assessment for Birds and Mammals’

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In 2021, EFSA has released the draft revised guidance document on ‘Risk Assessment for Birds and Mammals’ (hereafter GD). The GD aims to clarify methodologies and requirements for field studies. We review some of these requirements with particular focus on field studies that are aimed at assessing the ‘proportion of diet an animal obtained from pesticide treated area’ (PT).

According to the draft GD, it needs to be justified that the selected study area sufficiently represents a worst-case exposure scenario especially if the crop of concern is less abundant or if neighbouring habitats are diverse. However, it is not specified when crops are considered abundant nor how diverse habitats are defined.

Furthermore, according to the new requirement that at least ten consumer individuals are necessary for a valid PT study, the method that focuses on the selection of individuals for radio-tracking which represent the wider farmland population, is getting obsolete.

In contrast to the draft GD, we would like to recommend conducting multiple PT sessions on non-consecutive days for the same individual instead of a successive order. By adding additional days between single PT sessions, a better representation of intra-individual variation of PT estimates can be expected.

Additionally, in the current version of the draft GD, the crop attractiveness is calculated using the difference between PT<sub>90</sub> and PT<sub>50</sub>. The problem is that the same difference between both PT values can be attained by either mostly low or mostly high PT-values. This is the case when the crop is either used to a great extent or when the crop is only used to a small extend by most individuals. In order to solve this issue, we recommend to calculate the difference between the mean and PT<sub>50</sub>/PT<sub>90</sub>. In this case, the sign of the difference between PT<sub>50</sub> and mean indicates whether the crop is attractive or unattractive, while the difference between PT<sub>90</sub> and mean indicates – inversely proportional – the extent of attractiveness.



In summary, some aspects of the requirements for PT field studies are only rudimentary described in the draft GD and hold the potential to cause ambiguity among authorities on how to evaluate them. The provision of more details would help to avoid any uncertainty linked to the interpretation of given field study requirements. Furthermore, we hope that the integration of our proposed approaches regarding multiple PT sessions and the evaluation of crop attractiveness improves the draft GD.

#### 6.02.T-04 Risk Mitigation Measures of Pesticide-treated Seeds for Birds

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Treatment of sowing seeds with pesticides is a widespread agricultural practice that minimizes risks to operators and non-target species by reducing the amount of released pesticides but poses a particular risk to granivorous birds that feed on those seeds during the sowing season. It is therefore important to implement risk mitigation measures (RMM) to minimize the impact of this specific practice to birds. Over the last decade we have shown how granivorous birds are exposed in the wild to triazole fungicide through the consumption of treated seeds, and that this consumption can reduce their reproductive output. We evaluate three different, non-exclusive, RMMs to reduce the risk of triazole-treated seeds for granivorous birds associated with typical Mediterranean agro-ecosystems. The first RMM, focused on reducing toxicity, was the use of a non-toxic alternative treatment consisting of garlic essential oil encapsulated in chitosan nanoparticles (GEO/NPCH), whose antifungal activity and efficacy as seed treatment have been demonstrated. Toxicity tests showed no effects on exposed red-legged partridges associated with the ingestion of GEO/NPCH-treated seeds, which contrasts with the physiological alterations observed for conventional triazole treatments. A free-choice test showed no preference by partridges in the consumption of untreated, GEO/NPCH-treated or triazole-treated wheat seeds. The other two RMM were focused on reducing exposure by enhancing the availability of uncontaminated food. We supplemented recently sown fields with non-treated seeds and monitored the bird feeding behaviour using video-trapping cameras. Some species like pigeons occurred preferentially in supplemented fields, although they also feed in the sown treated seeds. Finally, we related the ingestion of triazole fungicides by hunted red-legged partridges to the diversity of landscape in the area where birds were hunted. Increased habitat diversity contributed to reduce the total intake of triazoles during the sowing season, likely because of a higher availability of natural food sources. Altogether our results reflect that RMM can be implemented at different levels. Habitat-based RMM are the most appropriate because they benefit different taxa and can be complemented with the use of non-toxic products like GEO/NPCH. Supplementation is however less efficient as it can act as an ecological trap by attracting birds that may end up feeding on treated seeds.

#### 6.02.P Bird and Mammal Risk Assessment: Now and Preparation for the Future

##### 6.02.P-We354 New EFSA Guidance Document update on Birds and Mammals: Industry view

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The Guidance Document (GD) on the risk assessment for birds and mammals (B&M) for pesticides (EFSA 2009) is now in the process of being officially updated by EFSA and will be finalised in 2022. SETAC Europe 2023 is an appropriate forum to share new information and to address controversial topics and/or the lack of harmonization in interpretation among regulators topics. Industry will contribute by sharing the experience from risk assessors who have been using the EFSA GD on a daily basis, highlighting those areas where the update should put more emphasis on or requires further guidance.

According to the impact analysis conducted by industry, it will not be possible to demonstrate acceptable risk for many of the currently registered active ingredients and plant protection products based on the screening step and the Tier I step of the exposure assessment, and this will trigger the need to generate a large amount of additional higher tier data. Numerous changes have been proposed in the new draft EFSA GD while its impact cannot be evaluated as protection goals are still pending. At least a 30-month period is considered necessary to generate reliable scientific data to address the new requirements. As for the bigger picture, significant legal, procedural, resource and knowledge gap, logistical and animal welfare concerns increase uncertainty. Focused actions by industry will contribute to minimize contradictory interpretations by risk assessors, increase the acceptance of, provide clarity and a path forward to a successful guidance. In the opinion of risk assessors from industry, the main refinement parameters that require more discussion before their implementation in routine risk assessment in the future are the following: the Benchmark Dose approach and the selection of the ecotoxicologically relevant reproductive endpoint, the use of the fTWA and agreed Tier 1 risk assessment, the selection of focal species of birds and mammals and further clarity or discussions on the higher tier options and modelling.

##### 6.02.P-We355 Avian Reproduction Studies: Experimental Design and Statistical Methodology

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Recent guidance from EFSA (2021) promotes the use of regression or benchmark dose methods to derive estimates of effect concentrations, BMD10, and BMD20 and their 95% lower confidence limits (BMDL10, BMDL20) in risk assessment of avian reproduction studies (OECD TG 206, OCSPP 850.2300). The previous study protocol called for a NOEC determination on selected endpoints using an experimental design with three application rates and a control with 18 replicate pens for each treatment level with each pen containing 2 or 3 individuals. Such a design is often inadequate for reliable dose-response and precise BMDx estimation.

A working group comprised of representatives from EFSA, USEPA, European Crop Protection Association, and independent scientists, collected a large database of studies conducted under existing and modified test designs to use as a basis for an extensive computer simulation study to explore alternative experimental designs and statistical methods that improve BMDx and BMDLx estimation. A secondary goal in this investigation is to keep the number of animals used to a minimum.

The analysis concludes that some responses from some studies cannot be fit reliably by most commonly used dose-response regression models and a NOEC must be used. EFSA guidance also acknowledges this possibility. Alternative experimental designs have been evaluated that indicate both adequate statistical power for NOEC determination in addition to adequate sensitivity of BMDx and BMDLx estimates for most study responses. The recommended designs require little or no increase in the number of animals compared to the older protocol, with an increased reliability of the hazard characterization estimates. Based on case studies and simulations, we recommend (1) generalized non-linear mixed models (GNLMM) for BMD estimation that allow for variance heterogeneity or overdispersion that is often observed in these studies and (2) model averaging in preference to model selection. Included in these recommendations are model acceptance and goodness-of-fit criteria and alternative ways to implement.

#### **6.02.P-We356 Experience to Date Deriving Endpoints for Birds and Mammals using the EFSA draft guidance (2021)**

*Helena Crosland and Amy C. Brooks, Cambridge Environmental Assessments (CEA), United Kingdom*

In 2021 the European Food Safety Authority (EFSA) released the draft of an updated bird and mammal guidance. The public consultation gave an opportunity to identify areas of potential concern – where changes in the guidance may result in less favourable outcomes to the risk assessment for Plant Protection Products, or where questions still remained as to how to apply the guidance. In the time since its release, experience has been gained in practically applying the draft guidance to the reproductive risk assessment for birds and mammals in terms of deriving toxicity endpoints.

Under the draft new guidance, ecologically relevant endpoints for both birds and mammals need to be derived. This requires reviewing the effects observed in the toxicity studies to determine which are population relevant and which aren't, with the guidance providing some advice on how to make these decisions. Additionally, benchmark dose modelling must now be performed to determine whether valid 10% effect levels (BMDL10) can be calculated and used in the risk assessment.

This poster aims to summarise our experiences to date, the challenges faced, the approaches chosen, and the uncertainties still present in the light of the updated guidance. Topics intended to be covered include the use of BMD modelling and the selection of ecologically relevant endpoints for both birds and mammals.

#### **6.02.P-We357 Software and Model Evaluations in Benchmark Dose Estimation (BMD) and BMDL for Avian Reproduction Studies**

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The draft of EFSA avian and mammal guidance recommends BMDL as the default Reference Point (RP) to replace LOEC/NOEC. BMDL is defined as the lower bound of the 95% confidence interval of the BMD, or benchmark dose, where the chemical dose or concentration produces a predetermined effect (10% in quantal data, and 5% in continuous data) in the response rate of an adverse effect (EFSA, 2017). Estimation of BMDL can be carried out with a range of different statistical approaches, whereby the choice of approaches can have notable influence on the final outcome. Agency-accepted software adopt different statistical approaches in the estimation of BMDL which can result in different conclusions for the same avian endpoint. The goal of this presentation is to evaluate the different statistical methodologies adopted by various software packages and assess the impact of on the final BMDL10 estimate.

Comparisons will be made amongst BMD software packages including but not limited to PROAST, BMDS, and ToxicR. Their impacts on the final BMD(L)10 estimates are investigated through comparing the software packages on a set of simulated datasets and case studies for quantal data. In simulated data, comparisons are made using the following metrics: 1) accuracy of the estimated BMD10 from each software package; 2) The normalized width of the confidence interval/credible interval from which BMDL10 is calculated; 3) identification of scenarios in which software fails to produce quality results. These comparisons provide some metrics to measure the robustness of each software.

Assessment across software packages and models confirms that through model averaging, BMD can be estimated with small amount of bias under scenarios with relatively good model fit. Even when there is a large discrepancy of BMD estimated between the model set, model averaging can still find the synthetic true BMD (from data simulation) with good accuracy. The subsequently estimated confidence intervals tend to be more sensitive to the model sets and model averaging method used. It could be sensitive to poor model fits which indicate the need for the ability to remove individual models when fit is clearly poor. Finally, we assess other factors like design bias and superiority of modeling quantal data over modeling transformed quantal data as continuous using the model averaging framework.

#### **6.02.P-We358 Draft Birds and Mammals Guidance, (2021): The Impact of fTWA Restricted Use on Long-Term Avian Risk Assessments Using LD50/10 Endpoints.**

**Kate Brougham**, *Environment, TSG Consulting, United Kingdom*

The regulatory approval of pesticides in the European Union requires applicants to conduct an ecotoxicological risk assessment for wild birds and mammals in accordance with the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438). However, the EFSA Working Group (WG) for Birds and Mammals is currently revising the EFSA, (2009) guidance document and, in September 2021, EFSA launched the public consultation on the updated draft. In the current EFSA birds and mammals guidance (2009), when toxic effects are considered to be caused by long-term exposure, a default time-weighted average factor ( $f_{TWA}$ ) of 0.53 (estimating TWA over 21-days and assuming a default half-life ( $DT_{50}$ ) of 10-days) is applied to the screening and Tier 1 assessment. The 2021 updated draft birds and mammals guidance proposes significant restrictions on the use of  $f_{TWA}$  in the long-term birds and mammals risk assessment including its use when the LD50/10 endpoint is used in the avian long-term assessment. We evaluate the impact of this restriction by considering the effect of removing the  $f_{TWA}$  from the risk assessments of compounds that have been evaluated by EFSA under the existing EFSA, (2009) birds and mammals risk assessment.

#### **6.02.P-We359 Laying Hen Studies and Time-to-effect in Reproductive Risk Assessments for Birds**

**Markus Ebeling<sup>1</sup>**, **Alex Blakey<sup>2</sup>**, **Manousos Foudoulakis<sup>3</sup>**, **Stephanie Plautz<sup>4</sup>** and **Magnus Wang<sup>5</sup>**, (1)Environmental Safety, Bayer AG - Crop Science Division, Germany, (2)Environmental Safety, Syngenta, United Kingdom, (3)Corteva Agriscience, Greece, (4)BASF SE, (5)WSC Scientific, Germany

For pesticides in the EU, the current reproductive risk assessment for birds is usually based on 21-d TWA (time-weighted average) concentrations in feed items (EFSA GD 2009).

The draft revision of the bird and mammal risk assessment guidance (2021) states that no TWA can be used for “effects on chick development” unless “significant evidence is provided to indicate that these effects are definitively linked to a long-term exposure”.

Effects on chick development can be induced by several pathways, e.g. changes in nutrient provisioning in eggs or in ovo exposure to toxicants. Contrary to mammals where the developing embryo is constantly provisioned by its mother, bird embryos only get what their mothers transfer into the egg before it is laid. Thus, it is the exposure of the mother for which the question of a link to long-term exposure is relevant in the TWA-context.

Ebeling et al (2022) have proposed to employ laying hen metabolism and residue studies for evaluating if the mechanism behind effects on chick development may be related to in-ovo exposure to residues transferred into the egg. In laying hen studies, adult chicken hens are exposed to a test substance over multiple days, typically by gavage. Some studies also include a depuration phase. The primary purpose of these laying hen studies is to inform the consumer risk assessment about residue levels in eggs, but these studies also inform about the kinetics of residue build-up in the eggs laid over the treatment phase.

When it can be argued (or demonstrated, for instance with an egg injection study) that the in-ovo exposure to such residues is the causative mechanism for effects on embryo development, then the time-to-plateau of the egg residues can be used to indicate the appropriate length of the TWA window. Where possible, PBK modelling may further refine such assessments and also allow direct incorporation of field-relevant exposure patterns as an alternative to a TWA-factor in the bird risk assessment.

In this poster we share the outcome of a data mining exercise in the regulatory study archives of our companies, and present examples for cases where the time course of egg residues is likely to inform an assessment of whether TWA can be applied to chick development endpoints.

#### **6.02.P-We360 Time Weighted Average Factor (fTWA) Assessments for Birds and Mammals under the Draft New Bird and Mammal Guidance: Experiences to Date**

**Amy C. Brooks<sup>1</sup>**, **Helena Crosland<sup>1</sup>** and **Alan Lawrence<sup>2</sup>**, (1)Cambridge Environmental Assessments, United Kingdom, (2)Independent Consultant

The potential risks to wild birds and mammals from foraging in areas treated with pesticides must be assessed in the EU under Regulation (EC) 1107/2009. Currently, the risk assessment is performed using the EFSA (2009) guidance, but this is in the process of being revised, with a draft new guidance being released by EFSA in 2021 and the final version expected to be released by the end of 2022. Within the draft guidance, a number of changes are proposed compared to the EFSA (2009) guidance, including the use of time-weighted average factors (fTWA) in reproductive risk assessments.

Under EFSA (2009), fTWAs could be used for reproductive risk assessments, with a default value of 0.53 being applied (relating to a  $DT_{50}$  of 10 days and an averaging period of 21 days) to dietary items such as crop plants, weeds, insects, etc where foliar uses of pesticides were proposed. The assumption behind this was that the toxic effects observed in reproductive toxicity studies were driven predominantly by long-term, rather than short-term, exposure, and therefore residue decline over time could be taken into account in the risk assessment. Conversely, if effects were expected to be driven by the initial maximum level of exposure, then taking into account residue decline over time (and thus a  $F_{TWA}$ ) would not be appropriate.

Under the draft new guidance (EFSA 2021), the fTWA is no longer used by default in the reproductive risk assessment for birds and mammals. Instead, an assessment must be made as to its appropriateness based on the effects observed in the mammalian and avian toxicity studies. The aim of this poster is to present our experiences to date in performing these assessments, highlighting potential issues and where further clarity of guidance is recommended where relevant.

#### **6.02.P-We361 A Population Genetic Analysis of Common Voles in Pome Fruit Orchards and Adjacent Meadows**

**Susanne Hein<sup>1</sup>**, **Felix von Blanckenhagen<sup>2</sup>**, **Tina Grimm<sup>3</sup>**, **Joachim Nopper<sup>1</sup>** and **Sonja Wich<sup>1</sup>**, (1)BASF SE, Germany,

(2)RIFCON, Hirschberg, Germany, (3)Rifcon, Germany

The common vole, *Microtus arvalis*, is often identified as a relevant focal species for which the chronic risk of the use of plant protection products is required to be assessed. In such a risk assessment, populations are the entity of concern. Consequently, to understand the chronic risk to common vole populations, it is essential to understand how populations are exposed.

We studied the population dynamics of common voles in orchards and adjacent meadows. During a field study in 2005, tissue samples from 554 live-trapped common voles were taken, DNA extracted from the samples and microsatellites of 10 loci analyzed. The frequency of exchange of individuals between habitats was assessed by analyzing genetic variability and gene flow within and between habitats in two consecutive seasons (spring and summer).

Habitat and seasonal differences in allele numbers and occurrence indicate a higher degree of immigration into orchards in spring than from orchards into meadows. In spring samples, allele numbers and the number of private alleles were higher in meadows than in orchards while this proportion reversed in the summer samples. On average, 51.8% of all new alleles observed in the summer samples of the orchard plots have previously been observed in spring samples from meadows, whereas 40.5% of newly observed alleles in late samples of meadow plots have previously been observed in spring samples from orchards. An analysis of likelihood of genotypes showed that 11.3% of all animals were likely to migrate from meadows into orchards whereas 7.2% were more likely to migrate into the opposite direction. The results indicate gene flow between vole (sub)populations from different habitats and consequently, an exchange of individuals between orchards and meadows. Depending on the habitat details, off-crop meadows can serve as starting point for common vole colonization of orchards in spring.

In conclusion, common vole (sub)populations in orchards are no distinct, closed populations but rather exchange with populations from adjacent habitats. Further, agricultural practices in orchards do not restrict the genetic variability of common voles.

Consequently, considering this exchange with the off-crop habitat is leading to a more realistic risk assessment. The results of this study can aid in determining suitable landscape compositions for more realistic risk assessments, e.g., when using individual based population modelling with common voles for risk assessments.

#### **6.02.P-We362 How to Demonstrate Representativeness of Field Studies using GIS – An Example with Common Vole in Grassland in Germany**

**Ines Hotopp<sup>1</sup>, Anja Russ<sup>2</sup> and Olaf Fuelling<sup>2</sup>, (1)tier3 solutions, Germany, (2)Tier3 Solutions, Germany**

The representativeness and transferability of field studies is often questioned by authorities. However, the current EFSA guidance on the risk assessment for birds and mammals (2009) and also the new draft guidance (2021) give no method or measure on how to prove that a study is representative or that its results can be transferred to another area. Geographic information systems (GIS) can be used to show that a study location is representative for a wider area, even a regulatory zone. Here, we give an example using the common vole in grassland in Germany. The analysis uses temperature and precipitation to describe climatic conditions, the proportion of different crop types to describe the agricultural landscape. As the study area, a region at the FNU Research Centre, North of Frankfurt (Germany), was selected. For the climatic conditions, the mean monthly temperature and the mean monthly precipitation sum were calculated. Agricultural landscape and occurrence of grassland were described using the EU crop map (2018) by d'Andrimont et al. (2021) calculating the proportion of the crop types. As common voles are ubiquitous in Germany, there was no need to analyse their occurrence. The parameters were calculated for 25 km grid cells overlaying the country and the study area. For each parameter, the mean of the study area, consisting of four grid cells, was compared to the whole of Germany by calculating the proportion of grid cells with similar conditions. The study area was representative of Germany for the chosen parameters with parameter values lying in the same range as values for most other grid cells. Climatic conditions, agricultural landscape and occurrence of the crop of interest were in a range common for Germany. For these parameters, the method chosen here worked well. However, a few factors should be considered when applying GIS methods: GIS data for specific crops, especially less common crops are hard to acquire. The resolution and the timeliness of data can cause problems. There are no standardized practices on how to analyse the representativeness of a study area. Using GIS is an option with many possibilities. However, the applicability of spatial data is still limited by data fragmentation and decentralized generation, because of competing interests and variety of formats. There is an urgent need for centralized access to data.

#### **6.02.P-We363 Availability of Pesticide Treated Seeds on the Soil Surface for Birds and Mammals After Drilling**

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For seed treatments, the current long-term tier 1 risk assessment for birds and mammals is based on the assumption that animals have *ad libitum* access to treated seeds. This is a highly conservative assumption as seeds are typically drilled into the soil, while only a small fraction of seeds may remain on the soil surface. Therefore, one of the key factors to realistically assess the risk from treated seeds to birds and mammals is their availability on the soil surface after drilling. To quantify the seeds' availability, De Snoo and Luttik (2004) reported soil incorporation rates in different crops, for different periods of the season and for different drilling techniques. These numbers are frequently used to conceptualize the risk for birds and mammals from exposure to treated seeds: Assuming that each and every seed available at the soil surface is eaten, the area which needs to be searched upon until a critical (toxic) level is reached, can be calculated. If this area is unrealistically large, compared to the foraging behavior of a species, the risk can be considered acceptable.

Due to advances in drilling technology, the seed incorporation efficiency has increased, leading to fewer numbers of treated seeds on the soil surface. Based on several field trials conducted after 2004 an updated database of incorporation efficiencies and exposure of treated seeds on the soil surface, respectively, will be presented, focusing on cereals, oilseed rape and sunflower.

Using this updated information will result in a more realistic and representative birds and mammal risk assessment for treated seeds.

#### **6.02.P-We364 Bird and Mammal Generic Field Studies – Early Application of Reliability Criteria According to the Draft Update to the EFSA Guidance Document**

**Christopher Taylor<sup>1</sup>**, Apostolos Koutsafitis<sup>2</sup>, Graeme Last<sup>1</sup>, Sarah Butler<sup>1</sup> and Steven Kragten<sup>3</sup>, (1)ERM Regulatory Services, United Kingdom, (2)ERM Regulatory Services, Netherlands, (3)Syngenta Agro, Germany

Under mandate from the European Commission, a revision to the EFSA Guidance Document for the Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12):1438) has been made and circulated for stakeholder commenting. Amongst the updated content in this revised Guidance Document is a much-expanded section on the assessment of 'Tier 3' generic field studies. This includes a dedicated set of evaluation criteria to assess the reliability and relevance of these study types. A dedicated 'Annex D' evaluation template is provided as part of the Guidance Document revision with the intent that future generic field study evaluation is conducted using this template.

The aim of the work presented here was to evaluate the suitability of this set of reliability criteria for the evaluation generic birds and mammals field studies. In total there are 21 distinct reliability criteria covering areas including general study information, study conditions, the organisms studied, the study methodology, and the statistical design and biological responses assessed. Each criterion is to be scored as one of 'fulfilled', 'not fulfilled', 'not reported' or 'not applicable' and, based on these, an overall reliability score assigned to the study.

The 21 reliability criteria were individually considered and further methodology to fully identify information required to fulfil each criterion was developed based on expert interpretation and judgement. In preparation and application of this methodology it was found that expanded methodology and 'weighting' of individual criteria was essential to the appropriate evaluation of each study. It was also found that some of the individual reliability criteria have no clear or straightforward answer as to what is sufficient for complete fulfilment and in these cases further expert judgement and/or clarification of the criterion is needed. Furthermore, the weighting of individual criteria to influence the overall reliability score for a study is not well instructed and can vary greatly depending on the extent of criterion fulfilment, as well as the objectives and primary endpoints derived from the study. In conclusion, further clarification and/or development of robust standard evaluation procedures is essential to most appropriately evaluate the reliability of these study types according to the new reliability criteria.

#### **6.02.P-We365 Residue Studies for B&M RA: Controlling the Uncontrollable**

**Helena Crosland<sup>1</sup>**, Gabriel Weyman<sup>2</sup> and Alan Lawrence<sup>3</sup>, (1)Cambridge Environmental Assessments, United Kingdom, (2)Gabe Weyman Consulting, United Kingdom, (3)Independent Consultant

In 2021 EFSA launched the public consultation on the draft updated guidance document on Risk Assessment for Birds and Mammals. This updated guidance included greater detail relating to the performance and evaluation of residue studies for the purpose of refining residue decline on food items for higher tier bird and mammal risk assessment. This is a welcome addition, as designing and performing such studies often raises a large number of complex problems to solve. However, experience to date of designing and monitoring such studies has highlighted many examples where reality does not conform to the guidance document requirements, or where the available guidance does not provide answers.

This poster aims to outline some of these issues, such as: locating sufficient amounts of a matrix for sampling (e.g. the natural lack of abundance of earthworms in some agricultural fields, particularly in Southern Europe); how to appropriately investigate residues in seeds; how to appropriately measure residues in plant matter when applying desiccating herbicides (and implementation in the risk assessment); what is an appropriate spatial and temporal distribution of trial sites; and how to deal with extreme weather events (what is the 'new normal' in the context of climate change?).

A consideration of potential solutions to these issues will be made, as well as reference to any useful information provided within the new draft guidance.

#### **6.02.P-We366 The Harvest Mouse (*Micromys minutus*) as Focal Species in the Risk Assessment**

**Olaf Fuelling<sup>1</sup>**, Ines Hotopp<sup>2</sup> and Anja Russ<sup>1</sup>, (1)Tier3 Solutions, Germany, (2)tier3 solutions, Germany

Hitherto, the in-field risk assessment of birds and mammals to plant protection products was considered protective for off-field populations. The EFSA's draft guidance on birds and mammals, though, underlines the need to consider some small mammal species in the risk assessment that are mainly found in the off-field area. In addition, a small granivore of 6 g should be considered in the crops with BBCH >39. One of the species that is thus becoming the focus of interest is the harvest mouse (*Micromys minutus*).

The granivorous harvest mice are widespread in Europe and Asia. As highly specialized stalk climbers, their original habitats were in reed beds and high grass fields. Secondly, they occur in cereal and beet fields, shrub-land interspersed with tall grasses and also in orchards. However, harvest mice are not commonly encountered in widespread surveys. They have a very patchy distribution, with 75 % of suitable habitat unoccupied with no clear justification for habitat colonization. Due to three-dimensional habitat use, harvest mice are usually underrepresented in standard small mammal traps set up on the ground. Also, the count of their elevated nests in tall vegetation is not an accurate measure to provide information on the actual population size, although detecting nests can be seen as prove of (recent) harvest mice occurrence.

Here, we discuss methods to identify off-field habitats and in-field occurrence of harvest mice that may be suitable for focal species studies with small granivorous species. Non-invasive methods like camera trapping, baited aerial tubes combined with species identification based on DNA from faeces samplings seem to be appropriate and timely methods for population studies of the harvest mouse to inform the risk assessment.

## 6.02.P-We367 Rabbits: Ecology, Management and Implications for Risk Assessment of Plant Protection Products

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The draft of the new Guidance Document on the risk assessment for birds and mammals in Europe (EFSA 2021) proposes to assess the risk of plant protection products (PPPs) to new generic model species (GMS) for many crop scenarios at Tier 1 with implications for the need of higher tier risk refinement.

CropLife Europe (CLE) conducted an impact assessment on 34 registered active substances covering 76 different representative uses (GAPs). The medium herbivorous mammal scenario had in almost 50% of all cases the lowest TER values for crop/species scenarios that failed. For risk assessments for the same use but following Guidance Document EFSA 2009 this was only the case for 20% of the failing scenarios. This indicates that under the new guidance document, the medium herbivorous mammal will often fail at tier 1 and will therefore often require refinement.

For higher tier risk assessment, the selection of focal species is a crucial step. The European rabbit (*Oryctolagus cuniculus*) is the medium herbivorous mammal with the lightest body weight in Europe and therefore potentially more exposed to PPPs compared to other species. However, based on scientific data, the relevant focal species has often been determined to be the brown hare (*Lepus europaeus*) and this has been accepted by several Member States. Because the new guidance document will result in many more failures of the medium herbivorous mammal scenario at Tier I (e.g. due to the increase in the number of scenarios for which medium herbivorous mammals are considered to be relevant, the use of BMDL10 instead of NOAEL, restricted use of the time-weighted average factor, assuming that herbivorous mammals feed on crops instead of weeds), the necessity to determine the relevant focal species in a higher tier risk assessment becomes increasingly important.

To better describe the relevance of the European rabbit in the risk assessment for PPPs, we here present the results of a thorough literature review on rabbits focusing on: European distribution, biology and population dynamics, feeding and habitat preferences, impact of agriculture on rabbits, impacts of rabbits on agroecosystems and rabbit population management in European farmland. Based on the information provided in the scientific literature, proposals will be made to facilitate the focal species selection for the medium herbivorous mammal scenarios for particular European regions as well as for particular crops and BBCH scenarios.

## 6.02.P-We368 Review of the Relevance of the Rabbit Prenatal Developmental Toxicity Study for Wild Mammal Risk Assessments

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The current EFSA guidance document for the assessment of risk for birds and mammals from exposure to plant protection products containing pesticides in accordance with Regulation (EU) 1107/2009, was first published in 2009 and the updated draft published in 2021 is currently under review.

The 2021 draft guidance outlines a tiered risk assessment scheme which includes evaluation of endpoints relevant for the maintenance of mammalian populations in the wild. The data on the relevant endpoints are derived from reproductive/long-term toxicological studies conducted in laboratory mammals as required for human safety assessment. Several studies on reproduction and development are routinely conducted for pesticides; all are generally conducted in the rat with one evaluation of prenatal development conducted using the rabbit. The rabbit is a required species because of its known susceptibility to some teratogenic chemicals which have no activity in the rat. However, the rabbit when used as a laboratory test species presents unique challenges affecting data study outcomes. Unlike the rat, individual rabbits do not respond in a consistent manner. In addition, it is worth considering that the rabbit has a unique digestive system and a known vulnerability to stress leading to outcomes which may be misinterpreted as toxicity.

That the rabbit is an important species for the detection of certain chemical teratogens is without doubt. But, is the rabbit a relevant species for the identification of systemic/maternal toxicity? Are all fetal observations that are considered human relevant also ecotoxicologically relevant?

In this poster we share the outcome of a novel analysis of the different parameters measured in the rabbit prenatal developmental toxicity study (OECD TG 414). Building on the 2021 draft EFSA Guidance document, we use these challenging questions and the answers to derive updated recommendations on the ecotoxicological (non-)relevance of the different study observations in this laboratory study as the basis for the endpoint for chronic wild mammal risk assessment.

## 6.02.P-We369 Seed Treatment Risk Assessment Scheme in the New EFSA Draft GD for Birds and Mammals: A Critical Review

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The current guidance document for birds and mammals (EFSA 2009) includes a risk assessment scheme covering exposure to treated seeds and emerging seedlings.

For the exposure to treated seeds, EFSA predicted a high failure rate at tier 1 and acknowledged that it is not possible to provide a standardised approach for a refined assessment. This has led to highly inconsistent evaluations by regulators for the same data set since the implementation of EFSA 2009.

In this presentation we highlight how the risk assessment for seed treatments in the new EFSA draft GD (EFSA 2021) compares to EFSA 2009.

For tier 1, we aim at presenting an impact assessment to evaluate how proposed changes in EFSA 2021 (e.g. no fTWA factor for treated seeds, use of BMD analysis on toxicity endpoint selection) affect toxicity-to-exposure ratios (TERs) compared to EFSA 2009.

For higher tier, we review proposed refinements in the EFSA draft GD, reflect the critical factors driving exposure of birds and mammals to treated seeds and seedlings under field conditions, and propose additional refinements to render the higher tier risk assessment more realistic.

Our preliminary analysis concludes that while EFSA 2021 includes positive elements for the emerging seedling scenario, there is no improvement over EFSA 2009 for the treated seeds scenario. With a more conservative tier 1, and fewer refinement options, we predict that even more products will be triggered into a weight-of-evidence (WoE) analysis for higher tier compared to EFSA 2009. At the same time, however, no detailed guidance is provided on how to conduct a WoE analysis and on how to evaluate WoE data from a regulator's perspective. This is considered problematic as WoE analysis are typically comprehensive, require significant resources, and rely on expert knowledge on agronomic practices and the ecology of exposed bird and mammal species. In the light of activities to reduce pesticide exposure in Europe, seed treatments present an effective technology to reduce exposure to non-target organisms. However, the proposed risk assessment scheme in the EFSA draft GD leads to high uncertainty for producers and farmers about the availability of this technology in the future.

We therefore promote an open discussion with key stakeholders and call for a timely revision by EFSA of the seed treatment risk assessment for inclusion in the final GD.

#### **6.02.P-We370 Reduced Reproductive Performance in Birds Feeding on Triazole-treated Seeds**

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During the last decades, there has been a decline in farmland bird populations across Europe. This regression has been attributed to agricultural intensification, which is characterized, among other components, by the use of pesticides. Triazoles are a group of fungicides used as seed coating treatments in crops, and wildlife associated with agro-ecosystems can easily come into contact with these compounds, especially granivorous birds that consume sown seeds. Triazoles are the most commonly found pesticides in digestive contents of red-legged partridges during the sowing season in autumn. Triazoles used for cereal seed treatment have been shown to affect the reproductive success in birds, reducing fertilization rate or brood size. The objective of this study was to measure reproductive effects on the red-legged partridge feeding on seeds treated with the formulated product Raxil Plus, one of the main products used for treatment of winter cereal seeds in Spain that contains tebuconazole and prothioconazole as active ingredients. We fed partridges with wheat seeds treated with 0% (control), 20% (low dose) or 100% (high dose) of the product labelled application rate. The animals were exposed during 20 days in late autumn, and reproductive parameters (i.e. fertilization, hatching and chicken survival rates) were later monitored in spring. In addition, gonadal size was measured in 12 partridges euthanized at the beginning and end of the breeding period. Fertilization, hatching and chick survival rates were significantly reduced in couples that had been fed with Raxil-treated seeds between three and six months before. These results agree with those found in other studies, which observed a 23% decrease in hatching rate and reduced brood size in partridges exposed to triazoles. In addition, the right testicle of birds that had consumed treated seeds was smaller than that of controls. The fact that triazole exposure in autumn may cause reproductive effects several months later supports the disruption of steroid-regulated processes as the mechanism of toxicity and reveals how the intensive use of triazole-treated seeds in agriculture could affect farmland bird populations through the impairment of their reproductive capacity.

#### **6.02.P-We371 Revised Residue Per Unit Dose Values for the Food Item “Seeds” At Late Crop Growth Stages for Use in Bird and Mammal Risk Assessments of Plant Protection Products**

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The draft of the new guidance document on the risk assessment for birds and mammals in Europe (EFSA 2021) provides default residue values, expressed as mg active substance/kg fresh weight, normalized to an application rate of 1 kg active substance/ha, for bird and mammal food items to be used in wildlife risk assessments. Some of these default ‘Residue-per-Unit-Dose’ (RUD) values for, e.g., monocot and dicot foliage, or fruits are based on large numbers of residue studies that were provided by industry and reviewed by EFSA.

The RUDs for crop and weed seeds are based on published literature (Fletcher et al. 1994) for residues measured in the plant category “leaves, leafy crops, forage crops and small seeds” and were last evaluated in 2002 (EC 2002). It was not possible for the authors to reproduce these RUD values that are still used today, since access to the data on individual residue trials, e.g., their location, the analysed matrix and crop, are lacking. Moreover, since current seed RUDs are based on an evaluation that was published in 1994, the values are likely based to a large extent on legacy chemicals that have long been withdrawn from the European market and may have been generated outside the EU. Altogether, data are of unclear relevance for regulatory purposes and current EU agricultural conditions.

To update the RUDs for the food item “seeds” at relevant crop growth stages of treated crops (BBCH  $\geq$  70) – i.e., not for (treated) seeds on the soil surface – we evaluated available residue studies that were conducted by industry for regulatory purposes in which residues on seeds were measured. Only data from GLP studies that were conducted in Europe were used. Trial data were

further processed when sampling took place directly after the last application. RUDs were then calculated using the maximum residue measurements after the last application accounting for the applied rate of the substance.

The analysis of the residue trials revealed significantly lower seed RUDs compared to the default seed RUDs given in the current EFSA guidance document (EFSA 2009), which are the same as in the new draft guidance document (2021) as well as the first guidance document on the risk assessment for birds and mammals in Europe (EC 2002). Consequently, new, and more realistic default RUDs for seeds are proposed that should be considered in future risk assessments.

#### **6.02.P-We372 Use of the Open-Source Software Open Systems Pharmacology Suite (PK-Sim® and MoBi®) in Environmental Risk Assessment**

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Environmental risk assessment of chemical substances covers the prediction and evaluation of the likelihood and magnitude of the potential adverse effects these substances may produce on wildlife, ecosystems and soil and water-based biological communities. Physiologically-based kinetic (PBK) modelling has emerged as a tool in several sectors (pharma, veterinary, medicine, cosmetics, and agrochemicals including pesticides) where they play a pivotal role for understanding and quantifying the chemical mode of action for risk assessment or precision medicine. PBK models are a mathematical representation of the animal body, describing the adsorption, distribution, biotransformation (metabolism) and elimination of a xenobiotic. PBK models can be built using different sources of data, such as *in vivo*, *in vitro* and *in silico* kinetic data. As new approach methodologies (NAMs) such as *in vitro* to *in vivo* extrapolation (IVIVE) are emerging also in environmental studies, the application of PBK modeling is key when it comes to interpretation and extrapolation of the data from *in vitro* toxicity assays to *in vivo* exposure doses.

The open-source software Open Systems Pharmacology Suite (OSPS, with tools PK-Sim and MoBi) is specifically tailored towards user friendly, robust, and workflow-supported (GUI-based) modelling and simulation. It combines the ease and efficiency of the GUI-based professional PBK tool PK-Sim with the customizability of the GUI-based mechanistic modeling tool MoBi (similar to Sim-Biology). PK-Sim provides physiology databases of multiple animal species and human populations (including maternal-fetal and preterm neonates). MoBi allows mechanistic modeling in all its detail and full user-based customization of the PBK models, allowing extensions of compartmental structures to any level of detail and the addition of complex kinetic/dynamic mechanisms for an integrated quantitative *in-vitro in-vivo* (QIVIVE) extrapolation framework. With this work, we discuss the use of the OSPS platform in support of regulatory decision making under the toxicology and environmental risk-assessment paradigm, showing the development of a generic bird PBK model. The strengths, gaps, uncertainties, and limitations of the platform will be discussed as well as how the validity and credibility of predictions/simulations is achieved. We aim to support a higher degree of confidence in the application of such models in a regulatory context.

#### **6.02.P-We373 What Does It Take to Make an Egg: A DEB Egg Laying Module for Birds Applied to the Bobwhite Quail**

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Understanding and managing risks builds on understanding the ecology and functioning of the system and/or organisms which are at risk. Birds inhabiting grasslands and wetlands, especially linked to agricultural or other commercial activities, are potentially exposed to harmful chemicals via water, food, or both. This positions birds as relevant model organisms for risk management, which, in turn, requires detailed knowledge on their ecology, metabolism, and relevant physiological processes. Particularly, growth, development, and reproduction are endpoints which are relatively easy-to-observe and to interpret. However, for many bird species the reproductive system builds up and major tissue remodelling happens at each reproductive season, coupled with increased food ingestion prior to and during reproduction. Despite research on this topic picking up, there is no clear scientific consensus on the energetic specifics behind these processes. We here used data available in the literature and reports to elucidate the energy fluxes linked to reproduction, by expanding a Dynamic Energy Budget (DEB) model for birds. The expansion of the standard DEB model consisted in a process-based explicit egg-laying module. We formulated and tested two assumptions for the energy allocation of extra assimilates observed in birds prior to and during the reproduction period. The first hypothesis assumed that the energy and nutrients are used directly for egg production. The second hypothesis assumed that the energy is mostly spent fuelling the increased metabolic costs incurred by building up and maintaining the reproductive system and ultimately by the egg laying process itself. We focused on one of the most studied and used bird species in risk assessment, the northern bobwhite quail (*Colinus virginianus*). Our results suggest that the second hypothesis is the more probable energy pathway. The model predictions capture well the development of the northern bobwhite quail and reproduce accurately most of the observed variability in egg size, egg-laying rate, and inter-individual physiology. As such, this model can be used as the physiological building block in the environmental risk assessment of birds exposed to anthropogenic stressors. Reliable models with an ability to predict physiological responses of individuals are relevant not only for experimental setups, but also for wild quail management and conservation.

#### **6.02.P-We374 DEB-TKTD Analysis of Avian Reproduction Study: The Bobwhite Quail Exposed to Fluopyram**

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Mechanistic modelling to analyse the effect of chronic exposure of chemicals to birds is rapidly gaining interest in the environmental risk assessment field. Lately, the new draft guidance document on risk assessment for birds and mammal from EFSA includes the possibility to use toxicokinetic-toxicodynamics (TKTD) models. In particular, the use of a TKTD module in combination with a physiological model like the Dynamic Energy Budget (DEB) model is recommended. In this study, we integrated a TKTD module into a DEB model with an egg-laying module for bobwhite quail (*Colinus virginianus*) to accurately predict the reproduction endpoints as well as the early development of chicks in both control and exposed situations. We calibrated the DEB module based on literature and control data and the TKTD module based on an avian reproduction study for the standard test species *C. virginianus* exposed to fluopyram. The model described generally very well the growth, development, and reproduction of the F0 generation for control and all different treatments. However, the model failed to capture accurately the wet weight of the F1 generation at hatch and at 14 days unless it was assumed that the mother transferred some of the contaminant into the eggs and that this impacted the offspring. This first analysis confirmed the need of an additional stress factor to capture the effect observed on the F1 generation. We therefore implemented an exploratory simplified TD module for the embryo and chick whereby the level of effect of the offspring is related to the level of contaminant in the mother. This additional module enabled the model to accurately capture the effects observed on the chicks of a mother exposed to fluopyram. Such approach highlights the added value of developing relevant process-specific modules (egg-laying in this case) and has the potential to make better use of existing and future data sets, providing means for a more accurate extrapolation of effects to birds from pesticide exposures in the field.

#### **6.02.P-We375 Challenges of the Assessment of Possible Synergistic Effects in Pesticide Formulations with More Than One Active Ingredient Using Acute Mammalian In Vivo Test Results**

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The draft EFSA guidance document on risk assessment for birds and mammals that was released in Sept 2021 requires an assessment of possible synergy for pesticide formulations containing more than one active ingredient. Acute oral toxicity studies using rats are often available for both active ingredients and formulations. However, these studies are typically conducted for classification and labeling purposes using the acute toxic class method (OECD 423) with a few female animals. The objective of this method is not the calculation of a definite LD50. Instead, the endpoint is expressed within a range of tested doses corresponding to the toxicity classes (e.g., 300 mg/kg bw < LD50 < 2000 mg/kg bw).

To assess whether additive or synergistic effects of several active ingredients in the formulation are expected, the EFSA Guidance document requires the comparison of LD50 values for the respective formulation. If the comparison between the predicted and experimental LD50 values, defined as MDR, deviate by more than a factor of 3, potential synergistic interaction between the ingredients is assumed and additional safety factors in the acute and chronic risk assessments are proposed.

Robust and definite LD50 values are required to generate reliable MDR values for a valid risk assessment. Otherwise, many conclusions of possible synergy are likely, where none actually exists, with associated risk assessment implications. We show exemplarily the challenges of calculating the MDR with non-definitive LD50 values. Innovative methods to evaluate possible synergy mechanism in wild mammals beyond MDR calculations using OECD 423 data for the formulation of interest are under exploration.

#### **6.02.P-We376 Challenges of Using Predicted Environmental Concentrations in Pore Water and Soil to Predict Secondary Poisoning Risk Assessment in Birds and Mammals**

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The bioaccumulative potential for substances with a log  $K_{ow} > 3$  and the risk of secondary poisoning for vermivorous birds and mammals is supposed to be assessed using the pore water approach as described in the revised draft GD (EFSA 2021). Within this new approach, the predicted environmental concentration in earthworms ( $PEC_{earthworm}$ ) is calculated by combining two different routes of exposure: direct exposure of earthworm tissue from substance residues in pore water ( $PEC_{pw}$ ) as well as by dietary exposure via consumption of soil ( $PEC_{soil}$ ). This is rendered possible by the newest revision of the soil exposure assessment as presented in EFSA (2017), where a  $PEC_{pw}$  is explicitly established along with  $PEC_{soil}$ . In this new framework,  $PEC_{pw}$  and  $PEC_{soil}$  are calculated by considering specific pedoclimatic scenarios to define realistic worst-cases, whether they are predefined scenarios (tier 1), or corresponding to a selected vulnerability level (95<sup>th</sup> percentile concentrations from spatialized simulations in the cropping area of interest in tier 2 and 3A). Specific scenarios must be established as the pedoclimatic conditions leading to realistic worst-case  $PEC_{pw}$  and  $PEC_{soil}$  are mutually exclusive:  $PEC_{pw}$  is maximum when the availability of the compounds in the liquid phase is high,  $PEC_{soil}$  when the substance remains in the modeled soil profile by adsorbing to the solid phase. This aspect is mainly driven by the Organic Matter (OM) content which governs the partitioning of the substance between liquid and solid phases of the soil, and its subsequent mobility. This results in vastly different modeling scenarios when conducting risk assessments either based on  $PEC_{pw}$  or  $PEC_{soil}$ : from tier 2 onward, the OM content at the 95<sup>th</sup> spatial percentile scenarios for  $PEC_{pw}$  and  $PEC_{soil}$  can differ by a factor of 4.8 to 5.8. Consequently, the combined use of  $PEC_{pw}$  and  $PEC_{soil}$  needs to be considered carefully. Using the direct outputs of the soil exposure assessment for  $PEC_{pw}$  and  $PEC_{soil}$  would lead to an unrealistically conservative earthworm exposure that would consider two mutually exclusive worst-case scenarios, which combination wouldn't be possible in the first place. In this poster we demonstrate the conservativeness of considering both worst-case  $PEC_{pw}$  and  $PEC_{soil}$  in comparison to more consistent and realistic  $PEC_{pw}/PEC_{soil}$ -pairs.

## 6.02.P-We377 Evaluation of Determining the Bioconcentration Factor in Earthworms in the Context of Secondary Poisoning – Dry Soil vs. Pore Water Approach

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Assessing the risk of secondary poisoning to earthworm-eating birds and mammals is a mandatory part of registration dossiers of plant protection products. The current EFSA Guidance Document for Risk Assessment for Birds and Mammals (2009) as well as the recently revised draft version (2021) describe two approaches to conduct the risk assessment: the dry soil and the pore water approach. An integral part of both approaches is the calculation of a theoretical bioconcentration factor (BCF) in earthworms that is then used to calculate a theoretical predicted environmental concentration (PEC) in earthworms. However, the calculations of the bioconcentration factor for the two approaches lead to widely different values. Within the dry soil approach, the BCF is calculated as  $(F_{\text{water}} + F_{\text{lipids}} * k_{\text{ow}}) / (f_{\text{oc}} * k_{\text{oc}})$ , where  $F_{\text{water}}$  and  $F_{\text{lipids}}$  are the volume fraction of water and lipids, respectively,  $k_{\text{ow}}$  is the octanol-water partitioning coefficient,  $f_{\text{oc}}$  is the fraction of organic carbon content in soil and  $k_{\text{oc}}$  is the organic carbon normalized soil adsorption coefficient. In the pore water approach, BCF is calculated as  $(F_{\text{water}} + F_{\text{lipids}} * k_{\text{ow}}) / \text{RHO}_{\text{earthworm}}$ , where  $\text{RHO}_{\text{earthworm}}$  represents the bulk density of an earthworm, which is equal to 1. Both  $k_{\text{oc}}$  and  $f_{\text{oc}}$  are not included in the equation for the BCF anymore and therefore the estimation of the BCF in the pore water approach is solely driven by the  $k_{\text{ow}}$  of a substance. Further, the term  $F_{\text{water}} + F_{\text{lipids}} * k_{\text{ow}}$  assumes a constant uptake of a substance into the earthworm, which seems biologically not applicable. For a more realistic BCF the relationship between  $k_{\text{ow}}$  and  $f_{\text{oc}} * k_{\text{oc}}$  might be therefore an essential part of the BCF calculation. We show the challenges of determining robust BCF values when using the BCF equation from the pore water approach in contrast to the BCF equation from the dry soil approach.

## 6.02.V Bird and Mammal Risk Assessment: Now and Preparation for the Future

### 6.02.V-01 Challenges of Benchmark Dose Analyses for Risk Assessors

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For benchmark dose (BMD) modelling as a method of parametric statistical modelling, certain prerequisites such as data following a certain distribution or having homogeneous variance must be fulfilled in order to be able to conduct the method correctly. Often, the fulfilment of these prerequisites is insufficiently checked or not checked at all. For example, in the case of biological data used for benchmark dose analyses, it is often not clear how they are actually distributed. Incorrect assumptions about the data distribution or even the selection of models that do not make biological sense can lead to the results of the analysis being significantly distorted. Moreover, routine data transformations can distort the data to such an extent that, in the end, a correct basic assumption is no longer fulfilled. The extent of the distorted results due to false assumptions can go in any direction: Too wide or too narrow confidence intervals of the BMD can be the result, whereby the corruption of the width affects both the robustness of the analysis and the BMDL (lower limit of the BMD confidence interval) as a reference point for the risk assessment.

The difficulty of handling data correctly to carry out valid BMD analyses already existed for the frequentist paradigm in the EFSA Guidance on the use of the benchmark dose approach in risk assessment (2017), still exists for the Bayesian paradigm in the updated EFSA Guidance (2022) and will be further explored here by showing examples of what can go wrong.

## 6.03.A Data-Driven Environmental Decision-Making: Generating Relevant Datasets for Regulatory Assessment of Endocrine-Disruption

### 6.03.A.T-01 New transgenic medaka model to detect disruption of thyroid signalling

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Endocrine disruption caused by chemicals has been considered as a major issue over the last few decades for both human and wildlife health. Identifying endocrine disrupting chemicals in order to limit their usage is therefore a priority. EU authorities has defined a testing strategy to determine the potential endocrine activity on EATS (Estrogen, Androgen, Thyroid, Steroidogenesis) axis. Regarding thyroid disruption, three OECD guidelines using *in vivo* non-mammalian models are validated, all of them are based on amphibian metamorphosis. In the most recent assay, the XETA (*Xenopus Eleutheroembryo Thyroid Assay*, OECD N°248), an amphibian (*Xenopus laevis*) transgenic line expresses a fluorescent protein which is quantifiable and proportional to thyroid axis activity.

To date no OECD test guideline based on fish are available for the detection of Thyroid Active Chemicals (TACs). We aim to fill this gap by developing a new test for the detection of TACs using medaka eleutheroembryos (*Oryzias latipes*).

This test involves a transgenic line, expressing Green Fluorescent Protein (GFP) under the control of the *thyroglobulin* promoter (the precursor of thyroid hormones). We first identify the medaka sequences presumably involved in the regulation of the expression of *thyroglobulin*. A transgene was then built by cloning this putative promoter upstream of the *gfp* coding sequence. Series of micro-injections were performed in one-cell embryos in order to develop transgenic lines. Screening the offspring reveals that four stable transgenic lines were obtained with different transmission rates but all having fluorescent thyroid follicles. The most promising line showed variation of total fluorescence when exposed to TACs, reflecting the negative feedback loop of Hypothalamic–Pituitary–Thyroid axis.

This novel transgenic medaka fish line *tg(tg:eGFP)* provides a promising tool for endocrine disruption assessment of chemicals in fish. Developing an assay based on this transgenic medaka offers the possibility to combine this assay with the REACTIV and/or

RADAR assays (OECD TG n°251). This strategy could lead to reduce the time, number of eleuthero-embryos and cost associated with EATS assessment in fish.

#### **6.03.A.T-02 Refining in vivo Fish Tests by inclusion of Thyroid Hormone System Sensitive Endpoints to improve Regulatory relevant Data**

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Several research projects and OECD member countries are working together to include thyroid hormone system (THS) sensitive endpoints into OECD fish test guidelines. Here we present an update of these efforts with emphasis on the results of the EU Horizon 2020 project ERGO and a proposal for a forthcoming OECD validation of selected endpoints. The ERGO project aims at breaking down the wall between mammalian and non-mammalian vertebrate regulatory testing by identifying, developing and aligning THS sensitive biomarkers and endpoints for linkage of effects between different vertebrate classes. A part of this work is dedicated to development and validation of potential THS sensitive endpoints in fish. Such does not yet exist but are highly warranted because fish tests are included as standard information requirements in many regulations. For example, OECD TG 210 the Fish Early Life-stage Toxicity Test (FELS) is required under the EU REACH regulation for chemicals imported or produced >100 tonnes per year and in the data requirement for active substances in plant protection products. With the addition of hazard classes for endocrine disruptors under the Classification, Labelling and Packaging Regulation (CLP), information on endocrine activity and adversity from standard fish tests become even more relevant. ERGO-generated fish data support project 2.64 (inclusion of THS sensitive endpoints in OECD fish Test Guidelines) which is on the OECD Work Program under the Validation Management Group for Ecotoxicity Testing (VMG-Eco) and co-lead by Denmark, Belgium and Germany, as well as project 1.35 of the OECD Adverse Outcome Pathways development programme workplan which is co-lead by the same countries and the US. ERGO project partners have developed and evaluated a number of endpoints sensitive to THS disruption, many of which are supported by well-established AOPs, using TG 210 as model for in vivo fish tests including the developmental period. Based on the outcome of seven TG 210 tests as well as a large number of embryonic tests, four endpoints have been selected for further evaluation. These endpoints are: 1) swim bladder inflation, 2) thyroid hormone (T3, T4) levels, 3) thyroid histopathology, 4) eye development and they are all supported by OECD VMG-Eco to bring to validation. We present the status of the work and suggest a way forward for OECD validation.

#### **6.03.A.T-03 An Amphibian Metamorphosis Assay Dietary Restriction Study: Lessons for Data Interpretation**

**Joseph Marini<sup>1</sup>, Jeffrey C. Wolf<sup>2</sup>, Valentin Mingo<sup>3</sup>, Stephanie Jamieson<sup>1</sup>, Lee Sayers<sup>1</sup> and James Robert Wheeler<sup>4</sup>,** (1)Smithers, (2)EPL, Sterling, (3)Corteva Agriscience, Germany, (4)Corteva Agriscience, Netherlands

The Amphibian Metamorphosis Assay (AMA) is a key in vivo endocrine screen to identify potential thyroid active chemicals. The Test Guidelines (TGs) in-study decision logic and associated guidance consider treatment-related effects on thyroid gland histomorphology to automatically result in the assay being considered positive for thyroid activity, independent of the direction of change or conflicting results in the other biological endpoints. European Union guidance for assessing endocrine disrupting properties of pesticides and biocides classifies the endpoints from the TGs as those that provide direct and indirect endpoints for the evaluation of thyroid activity. Here, it is assumed that accelerated and asynchronous development, as well as histopathologic effects are specific for a chemical-induced thyroid hormone interaction in the absence of overt toxicity.

The goal of this study was to evaluate how a non-chemical stressor, dietary restriction, might affect the outcome of the AMA, and in particular, developmental effects that are currently considered to be mediated specifically through the thyroid endocrine axis. To this end, an AMA was conducted with 5 different feeding regimes equivalent to 50 (reference), 30, 20, 10 and 5% of the TG recommendation. All endpoints were evaluated and their specificity for the determination of thyroid activity was assessed. Statistically significant reductions in growth and development were observed between the 50% and 30, 20, 10 and 5% rations. Findings in the thyroid glands included ration-dependent reductions in the prevalence and severity of follicular cell hyperplasia and follicular cell hypertrophy, and a ration-dependent increase in the prevalence and severity of thyroid atrophy in tadpoles of the 10% and 5% groups.

The results indicate that treatment-related histopathologic effects in the thyroid are not necessarily specific for thyroid endocrine activity and can alternatively be induced by non-hormonal factors as the initiating event. Therefore, the interpretation of data from AMA studies should be adjusted accordingly. We recommend the decision logic presented in the TGs should be changed to reflect an element of agreement between the thyroid histopathology findings and direction of change of the apical endpoints, to conclude on the thyroid endocrine activity of a test substance.

#### **6.03.A.T-04 Amphibian Studies to Investigate the ED Properties Through the Thyroid Modality: A Comparison of Their Statistical Power**

**Simone Rizzuto, Alessio Ippolito, Aude Kienzler, Alberto Linguadoca, Laura Villamar-Bouza and Maria Arena, European Food Safety Authority, Italy**

The ECHA/EFSA ED Guidance, in accordance with the OECD Guidance 150, recommends using specific amphibian studies to investigate the ED properties through the Thyroidal (T) modality, based on the available evidence indicating that amphibian metamorphosis is under the control of the hypothalamic–pituitary–thyroid (HPT) axis, and that regulatory toxicity tests on amphibians are standardized and well consolidated. Currently, two validated amphibian standard toxicity tests are available: the amphibian metamorphosis assay (AMA, OECD TG 231), and the larval growth and development assay (LAGDA, OECD TG 241). An extended AMA (EAMA) assay was developed until reaching stage NF62.

AMA studies are developed to conclude on whether a tested substance shows endocrine activity. If AMA is positive, a mode of action should be postulated and additional data should be granted to investigate ED adversity, by providing a level 4 CF study (i.e., LAGDA), in line with the general agreement that level 3 studies (AMA) should serve to screen for endocrine activity and not for the derivation of a precise endpoint for adverse effect (NOEC/EC<sub>x</sub>). This raises a potential concern on whether the statistical power of level 3 tests may be insufficient to conclude on potential adverse effects.

A recurring debate is whether conducting a LAGDA is necessary to confirm or clear off concerns identified by a positive AMA. Critical views suggest that both AMA and LAGDA perform a larval assessment of key diagnostic responses for thyroid effects, questioning the added biological value of LAGDA. The direct value in terms of statistical power is also questioned, since the number of tested animals equal across AMA and LAGDA. A similar common debate in the regulatory context is whether it is safe to exclusively rely upon the results of AMA studies to draw conclusions on endocrine adversity. Specifically, a key, unexplored aspect of this debate is whether null results can be deemed conclusive of the absence of effects, considering that aspects of the AMA design may limit our ability to statistically detect effects on diagnostic responses of the T-modality. Here we address this knowledge gap by testing the hypothesis that the statistical power for key diagnostic responses of the T-modality differ across different study designs (i.e., AMA, LAGDA and EAMA). Our results may improve our knowledge and understanding of the testing strategy for the regulatory ED assessment of pesticide active substances.

#### **6.03.A.T-05 Amphibians in Thyroid Disruption Assessment: From Testing Strategies to the Identification of Thyroid-related Effect Patterns**

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Metamorphic development of amphibians is controlled by thyroid hormones and test guidelines (TGs) have been developed by the US EPA and OECD with *Xenopus laevis* to evaluate thyroid-mediated activity and adversity. Within the OECD Conceptual Framework for testing and assessment of endocrine disruptors, the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) and the Amphibian Metamorphosis Assay (AMA) are level-3 screening assays providing data about selected endocrine mechanism(s)/pathway(s). The AMA also includes apical endpoints that can inform on potential adversity, especially when the test is run according to a fixed termination stage design (i.e., the Extended AMA or EAMA). The Larval Amphibian Growth and Development Assay (LAGDA) is an OECD level-4 assay that has a limited validation dataset for the assessment of the thyroid modality and does not always allow for the distinction between endocrine and non-endocrine modes of action. Since the LAGDA is not typically conducted for the initial assessment thyroid-mediated activity and is not generally considered a reliable test for investigating thyroid-mediated adversity, testing strategies generally rely on a combination of the XETA, AMA and/or EAMA, as well as *in vivo* mammalian mechanistic studies and *in vitro* assays focusing on specific thyroid molecular targets such as the sodium-iodide symporter (NIS) and thyroperoxidase (TPO). In certain conditions, partly described in Annex A of the ECHA-EFSA guidance for the identification of endocrine disruptors, animal testing can be avoided by combining the XETA with selected *in vitro* assays and an understanding of the mammalian toxicology dataset. This presentation will describe how OECD level-3 amphibian assays can be combined with *in vitro* and other *in vivo* data into integrated test strategies for regulatory assessment of thyroid-mediated activity and adverse effects. In the AMA and EAMA, there is an interplay between endocrine activity and adversity endpoints. The direction, concomitance, and severity of changes of the developmental and histological parameters measured are therefore essential to the identification of endocrine-related effect patterns specific to the thyroid modality. The decision logic of OECD TG 231 should be changed to reflect an element of agreement between thyroid histopathology findings and direction of change of the apical endpoints.

#### **6.03.B Data-Driven Environmental Decision-Making: Generating Relevant Datasets for Regulatory Assessment of Endocrine-Disruption**

##### **6.03.B.T-01 Evaluation of Interactions of Endocrine Disrupting Chemicals (EDCs) with Human and Zebrafish Nuclear Receptors (NRs) using Reporter Cell Lines**

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Reporter gene cell lines expressing Nuclear Receptors (NRs) are widely used to study the effects on human and wildlife of Endocrine Disrupting Chemicals (EDCs), substances that can interfere with their endogenous hormonal pathways. Given the large number of chemicals to be studied in the environment, the development of *in vitro* tools makes it possible to significantly reduce animal testing. However, as the OECD guidelines for EDC testing require the use of reporter cell lines expressing only human NRs, the scientific gap regarding the effects of EDCs on wildlife is filled by extrapolating mammalian data to other species. Our group has developed cell lines expressing reporter genes of human (h) or zebrafish (zf) NRs with the aim of studying interactions of chemicals with NRs of different species and improving the regulatory testing approaches. These cell lines can be used to study in a species-specific manner hundreds of chemicals for their endocrine-disrupting properties towards different molecular endpoints. Our method consists of transfecting the NR reporter gene of interest together with a luciferase gene whose expression is under the control of the receptor. In this way, it is possible to evaluate the effects of chemicals on the NR as they are able to modulate the luminescence signal. Our results showed that, depending on the NR studied, interspecies differences are more (e.g. Progesterone Receptor, Peroxisome X Receptor) or less pronounced (e.g. Androgen Receptor, Glucocorticoid Receptor). Furthermore, as part of biological monitoring, NR-expressing cell lines can be used to analyse environmental extracts (e.g. water and air samples) to assess the effects of environmental chemicals on NRs in addition to chemical analysis. Our results showed that

the analysis of environmental samples with NR-expressing cell lines confirmed some interspecies differences observed with pure chemicals and allowed us to choose the most appropriate cell lines according to the purpose of the research. In conclusion, our work broaden knowledge on the effects of several environmental chemicals on different NRs and species. Biological assays using reporter cell lines expressing NR of different species can be used as a screening tool to assess the effects of numerous EDCs and at the same time greatly reduce animal testing, which is particularly important in view of the 3R principle.

### 6.03.B.T-02 Nuclear Receptor-Mediated Endocrine Disruption in Marine Invertebrates: A Case Study with the Mediterranean Mussel *Mytilus galloprovincialis*

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Endocrine Disrupting Chemicals (EDCs) are a global threat to populations of marine invertebrates. Although it is well-known that the main action of EDCs is to mimic endogenous hormones and bind to their Nuclear Receptors (NRs), the scarce knowledge of NRs functions and transactivation dynamics in most invertebrate clades prevents the understanding of NR-mediated endocrine disruption in these organisms.

In this light, the present work characterized the NR superfamily of the Mediterranean mussel *Mytilus galloprovincialis* (Mg), investigating potential developmental functions, ligand affinities and susceptibility to endocrine disruption.

MgNRs were isolated from the mussel genome, their developmental expression dynamics were obtained from RNA-seq data, and expression patterns were assessed by In situ Hybridization (ISH) and Hybridization Chain Reaction (HCR). Pharmacological manipulations largely focused on MgRXR (Retinoid X Receptor) and its heterodimeric partners (HPs) using 9-cis-Retinoic Acid as positive control (RXR agonists), UVI3003 (RXR antagonist) as negative control, and Tributyltin (TBT) as model EDC.

36 canonical MgNRs were identified with most of them fitting in the current nomenclature and classification. All MgNRs were dynamically expressed at different developmental stages, in particular the members of NR subfamily 1, 2, 3 and 4. Most MgNRs showed discrete localization in neurogenic and shellbiogenic tissues suggesting a pivotal role of the superfamily in governing mussel larval morphogenesis.

MgRXR pharmacological modulations with both TBT and RA caused developmental arrest at the pre-Veliger stage. Such adverse effect could be consistently reversed by co-exposure to RXR antagonist hence demonstrating the specificity of the phenotype to MgRXR disruption.

MgRXR modulation also inhibited multiple elements of the neuroendocrine pathway governing early shell biogenesis thus showing a potential link between NRs and the neuroendocrine signaling in a mollusk.

Interestingly, TBT and RAs did not induce the same neural phenotype. Given the subordination of RXR transcriptional activity to the one of its HPs, it is parsimonious to theorize that the binding of both chemicals to MgRXR might not necessarily induce the transactivation of the same heterodimers.

Altogether, the findings of this work represent a step forward in the understanding of NR signaling and susceptibility to endocrine disruption in bivalve mollusks.

### 6.03.B.T-03 Are Changes in Vitellogenin Concentrations in Fish Reliable Indicators of Chemical-induced Endocrine Activity?

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The egg-yolk precursor protein vitellogenin (VTG) is widely used as a biomarker for endocrine-mediated activity via estrogen (E), androgen (A) or steroidogenesis (S) pathways. Reliable and robust measurements of VTG are therefore required to support decision-makers in the regulation of chemicals for their environmental endocrine activity. VTG concentrations are inherently variable and equivocal results from fish studies are common and can lead to additional fish testing to refute or confirm a previous result. A comprehensive review of VTG data was conducted, including 98 substances (general chemicals as well as substances with known endocrine activity) focusing on regulatory test species; namely fathead minnow, zebrafish and Japanese medaka. The review confirmed the high variability in VTG response with coefficient of variations for interlaboratory variation being between 201-543% in males and 76-206% in females. VTG induction in male and female fish was tabulated for substances with E receptor (ER) agonist activity (based on potency estimates from the ER bioactivity model) and substances which inhibited VTG induction based on aromatase inhibition (AC50). Substances with a higher ER agonist bioactivity score were more likely to induce VTG in males to levels which were greater than the female control response and/or to induce VTG in females (in at least one study). Most of the substances which caused inhibition of VTG were inhibitors of aromatase *in vitro*. Exceptions to this were most notably for substances with multiple mechanisms of action. There were also a few examples of substances which did not have evidence for endocrine activity *in vitro* causing repeatable, concentration-dependent effects on VTG, and these were substances with active metabolites, or which potentially affect steroidogenesis other than via aromatase. To increase confidence in the VTG measurement we recommend improvements to the consistency of VTG analysis, and greater transparency in reporting of VTG measurements (including quality control criteria for assay performance). This review supports the application of New Approach Methodologies (NAMs) by demonstrating that endocrine effects in *in vitro* studies with mammalian cell lines are predictive for *in vivo* effects on VTG in fish, suggesting that NAM data could be used more broadly in decision making to help reduce animal testing.

### 6.03.B.T-04 Population Models in a Hazard Assessment: Population Relevance of the Effects of Endocrine Active Substances

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All agrochemicals being authorised for use in the EU need to be assessed against the criteria for being identified as an Endocrine Disruptor (ED), namely whether they have an adverse effect that is the result of an endocrine-mediated mechanism. For non-target organisms, the adverse effect being considered is at the population level (e.g. population abundance). However, those endpoints reported in recommended studies on endocrine active chemicals are commonly at the individual level (e.g. significant effects on fecundity in fish). Whilst the ECHA-EFSA guidance on how to assess for an EDC indicates that population modelling may be used to link observed effects on individuals in laboratory studies to population-level effects relevant for the assessment criteria, it provides no practical guidance on how this might be done. Here we present how population modelling could be used as an initial screening step for assessing the population relevance of adverse effects observed on individuals in the laboratory. Using the Adverse Outcome Pathway (AOP) concept, this method could be applied to any molecule being considered under EC1107/2009 for use in the EU.

Results from three fish population models available from the literature: zebrafish (*Danio rerio*), stickleback (*Gasterosteus aculeatus*) and brown trout (*Salmo trutta*) are presented. Selected apical endpoints assessed in higher level ED studies, namely fecundity, fertilisation rate, sex ratio (male and female skew), behaviour and growth, were implemented both individually and in combination at different magnitudes. We used a magnitude of effect of 10, 20, 50 and 90% change in an endpoint to assess both population abundance and biomass against the control simulations (no effects imposed on any endpoint) using the two criteria from EFSA.

Aim of this work is to demonstrate how population modelling can be used to extrapolate the relevance of ED-mediated effects on individuals to whole populations, thereby better linking the assessment endpoint (adverse effect on population abundance or biomass) with the protection goal (population) of an ED assessment under EC 1107/2009 and Commission Regulation (EU) 2018/605.

### 6.03.B.T-05 AOP Linking RAR/RXR Overactivation to Feeding Disruption

**Audrey Phan**, Marie Smutna, Aleksandra Sokolova and Klara Hilscherova, RECETOX, Masaryk University, Czech Republic  
Retinoid signaling disruption has been overlooked in regulation. However, in the last few years, there is an increasing interest in retinoid-disruption related adverse effect in (eco)toxicology because of regulatory needs. Indeed, the known conserved mechanisms of retinoid signaling across species and the environmental occurrence of retinoids are contributing to the potential high risk when retinoid signaling disruption occurs.

In the line of AOP development, we focused on a specific adverse outcome, craniofacial malformation. After conducting a weight of evidence analyses, a lack of data linking craniofacial malformation to regulatory endpoints was identified. Here, we focus on linking malformation to feeding disruption in early larval stages and survival at late larval stages. As posterior swim bladder inflation could also play a major role in buoyancy and thus feeding behavior, we also assessed this endpoint. Results show that the derived EC50 for craniofacial malformation, EC50 for no inflation of the posterior swim bladder, and IC50 for feeding disruption were comparable. The data indicate that in the context of teratogenicity induced by RAR overactivation, a combination of effects occurring in parallel leads to decreased feeding. We also link the feeding behavior disruption at the early larval stage (7dpf) to mortality at the later larval stage (14dpf). 14 dpf zebrafish larvae showed ~90% mortality at ATRA 1µg/L regardless the exposure time: 6-48hpf; 6-72hpf; 6-120hpf.

To conclude, we demonstrated quantitatively that the teratogenicity induced by the overactivation of RAR/RXR leads to a disruption in feeding behavior and mortality. By adding a regulatory-relevant endpoint to a putative AOP and by providing quantitative data, we bring together data that could fit a future framework implementing AOP into risk assessment.

### 6.03.P Data-Driven Environmental Decision-Making: Generating Relevant Datasets for Regulatory Assessment of Endocrine-Disruption

#### 6.03.P-We378 Investigations into the Induction of Thyroid Hormone Liver Metabolism in Zebrafish (*Danio Rerio*) Embryos

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Hepatic TH metabolism consists of two major pathways: Deiodination and conjugation. The conjugational pathways comprise glucuronidation by UDP-glucuronosyltransferases (UGTs) and sulfation by sulfotransferases (SULTs), both of which increase water solubility and thus facilitate biliary excretion. In addition, sulfated THs are also inactivated by deiodinase 3 at a much higher rate than the parent hormone itself. Expression of conjugation enzymes is influenced by a variety of nuclear receptors, such as the aryl hydrocarbon receptor among others. These receptors accept a wide range of exogenous agonists, and their activation generally leads to an increase in the expression of enzymes involved in the metabolism of xenobiotics. As an off-target effect, UGT and SULT expression can also be increased to levels that negatively affect TH homeostasis. While disruption *via* this pathway has been observed in multiple mammalian species, it has so far not been explored in detail in fish. In the context of the 3R principles, it would be highly beneficial to determine whether this mechanism leads to similar outcomes in fish and other lower vertebrates. Specifically, the applicability of zebrafish (*Danio rerio*) embryos as a model predictive of the identification of altered hepatic TH clearance would help to reduce animal testing in mammals. To properly evaluate the induction of hepatic TH metabolism in zebrafish, multiple endpoints need to be investigated at different steps in the pathway, ranging from molecular key

events to physiological changes. In addition, multiple model substances, which are known agonists of the nuclear receptors involved in UGT and SULT expression, need to be tested to determine the impacts of different molecular events. For this, both a wildtype as well as a transgenic zebrafish line with fluorescent thyroid follicles were exposed. As endpoints, gene expression analysis of multiple thyroid- and metabolism-related genes, fluorescence intensity and area measurements of the transgenic zebrafish thyroid follicles as well as eye histopathology were selected to cover mechanistic events as well as biologically relevant apical endpoints in development. Taken together, results of these experiments provide insight into the mechanisms of and effects on hepatic TH metabolism in zebrafish embryos, which can then be compared to literature data on other vertebrates to establish both similarities and differences between species and vertebrate classes.

#### **6.03.P-We379 OECD Endorsement of an AOP Network of 5 AOPs Linking Thyroid Hormone System Disruption to Impaired Swim Bladder Inflation in Fish**

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Over the last 10 years, through the success of several projects, five adverse outcome pathways (AOPs, AOP-Wiki IDs 155-159) have been developed linking thyroid hormone system disruption (THSD) to adverse effects on swim bladder inflation and survival in fish. The ECO20-UA project (2013-2016), funded by the Cefic Long-Range Research Initiative, investigated whether the AOP framework could provide a mechanistic basis for predicting chronic fish toxicity using non-animal alternatives. The AOPs were developed up to the point that they allowed selection of key events and development of alternative assays to identify thyroid hormone system disrupting chemicals (THSDCs). The follow-up ECO20.2 project (2016-2018) applied the alternative assays developed during ECO20 to a set of selected compounds, predicted acute and chronic toxicity, and performed Fish Embryo Acute Toxicity (FET) and chronic Fish Early-Life Stage (FELS) toxicity testing to validate the predictions. In the ERGO project (2019-2023), funded under the Horizon 2020 programme, the AOPs were completed and submitted for review by OECD. The AOPs have now been endorsed by the Working Group of the National Coordinators of the Test Guidelines program (WNT)/Working Party on Hazard Assessment (WPHA) and will soon be published in the OECD Series on Adverse Outcome Pathways. We are now working on a broader network of THS AOPs to support cross-vertebrate extrapolation of mechanisms and effects of THSDCs for informing both on environmental and human health. Pre-validation of fish assays is also ongoing within ERGO. The endorsement and publication of these AOPs by OECD supports the addition of new endpoints to existing OECD fish Test Guidelines (TGs). This is facilitated by the close integration of AOP and TG development at the OECD level. Project 1.35 (Lead: BE, partners: US, DK, DE) on the OECD AOP development programme workplan overseen by EAGMST was initiated in 2015 and comprises the development of AOPs for THSD in fish. Project 2.64 (lead: DK, partners: BE, DE) on the OECD work plan for the Test Guidelines Programme overseen by VMG-Eco was initiated in 2019 and uses the AOPs as support for adding thyroid-sensitive endpoints to existing fish TGs. Such refined TGs will improve the evaluation of THSDCs and will be included in approaches for extrapolation of THSD effects across mammalian and amphibian species. The contents of this abstract neither constitute, nor necessarily reflect US EPA policy.

#### **6.03.P-We380 Potential Endpoints to Identify Thyroid Hormone System Disruption in Zebrafish Embryos**

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Identifying thyroid hormone system disruptors (THSD) is warranted because of the crucial role that thyroid hormones (THs) play in the normal development of vertebrates. Thus, selection of a sensitive animal model and identification of suitable endpoints for detecting THSDs are key for guiding regulatory decisions. OECD fish test guidelines so far lack TH system sensitive endpoints. However, the TH system regulates a wide range of measurable molecular and morphological endpoints in fish, including swim bladder inflation, thyroid- and eye-related gene expression, and the development of thyroid follicles and eyes. This study aimed to investigate whether these endpoints are suitable for detecting THSD in the zebrafish (*Danio rerio*) embryo by using the existing OECD test guideline 236 (FET, fish embryo toxicity test). We performed experiments with multiple THSDs with different modes-of-action, such as propylthiouracil (PTU), potassium perchlorate (PCL), iopanoic acid (IOP) and the natural thyroid hormone triiodothyronine (T3). The basic protocol of OECD TG 236 was followed, and different TH system sensitive endpoints such as gene expression, detection of cellular changes in the eyes (e.g., cell size, cell layer structure and organization, number of photoreceptors, pigmentation etc.), as well as morphological changes (e.g., alterations of thyroid follicular morphology in transgenic fish) were added. For thyroidal analysis, transgenic zebrafish embryos expressing the reporter gene tg:mCherry were used. Results demonstrate that zebrafish embryos react sensitively to THSD. Changes in the expression of the eye- and thyroid-related genes in the zebrafish embryos were observed and different modes of THSD resulted in various alterations of thyroid follicle morphology and fluorescence intensity in the transgenic line. Furthermore, strong alterations of eye morphology and cellular structure, combined with changes in photoreceptor patterning, could be recorded by (immuno-)histopathology. Results confirm growing evidence that zebrafish embryos are sensitive to THSD treatment and represent a promising tool for assessing TH system-related effects in fish. The FET protocol according to OECD TG 236 proved to be a suitable basis for implementing TH system sensitive endpoints in fish, as it covers important stages of early development, many of which are regulated by THs.

#### **6.03.P-We381 The Early Life Stage Amphibian Thyroid Assay (ELSATA), a Thyroid-Focused Alternative to the Larval Amphibian Growth and Development Assay (LAGDA)**

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The Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241) was designed as a higher tier *in vivo* assay meant to provide data on adverse effects of substances on thyroid-mediated endpoints. As a level 4 test within the OECD Conceptual Framework on Endocrine Disruptors Testing and Assessment, it is a final step of testing in the ECHA-EFSA testing scheme for the thyroid modality, to be utilised in case a Level 3 Amphibian Metamorphosis Assay (AMA, OECD TG 231) indicates thyroid activity of a substance and thus, the need for the generation of additional data. However, the LAGDA is not limited to generating endpoints related to the thyroid modality, as the second half of the study is focused on the estrogen, androgen and steroidogenic (EAS) modalities. Considering that the triggering of a LAGDA is based on concerns around the thyroid axis, questions have been raised as to the value of this additional 10-week period in the study for identifying any additional effects related to (anti)thyroidal activity of substances. Initiation at NF stage 10 considers embryo-larval and pre-metamorphic development, already providing a means for assessing potential alternative modes of action and discriminating non-endocrine from endocrine toxicity. Additionally, for the purpose of an assessment on the thyroid modality, generating unrelated data causes needless animal welfare concerns. In an effort to focus on the core strength of LAGDA, we have investigated a modified version of the study which only considers endpoints related to the thyroid modality. In doing so, animal welfare is emphasized, as fewer individuals are used for a shorter total duration, crucially without decreasing the amount of thyroid-specific data generated. Results from the evaluation of this Early Life Stage Amphibian Thyroid Assay (ELSATA) will be presented.

#### **6.03.P-We382 Use of *Xenopus laevis* Developmental Stage-matched Control Data in Amphibian Metamorphosis Assays to Assess Inter-laboratory Variation in Endpoint Data**

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The analysis of continuous quantitative *Xenopus laevis* endpoints such as snout-vent length (SVL), hind limb length (HLL), hind limb length normalized by snout-vent length (nHLL), and wet weight is performed to assess statistically significant differences between the negative control and active substance treatments in the Amphibian Metamorphosis Assay (AMA) for the detection of potential thyroid modes of action according to Guidelines OECD 231 and OPPTS 890.1100. However, the current statistical analysis of these parameters at Day 7 and 21 is routinely performed by using the replicate means or medians of the individual endpoint values without taking into consideration the different stages of development within each control or treatment replicate. Furthermore, these endpoints are well known to be directly impacted by developmental stage in tadpoles and so using stage-matched data may have an impact in the interpretation of the biological results. This is also the case for tadpole thyroid gland histopathological findings, where it is already recognized in both Guidelines that the most appropriate approach is to use NF developmental stage-matched tadpoles for evaluation. Consequently, individual control HLL, nHLL, SVL, and wet weight values for the Day 7 and 21 termination events from multiple Contract Research Organizations have been extracted and evaluated according to NF developmental stage. Stage-matched and non-stage-matched data were also evaluated against several study parameters such as food and water quality. The analysis presented here aims to develop a NF developmental stage-matched Historical Control Database that allows the assessment of potential impact in the outcome of statistically significant results of these thyroid screening parameters, as well as the variability of NF developmental stage-matched control data across different GLP AMA-performing laboratories.

#### **6.03.P-We383 Impacts of Non-endocrine Factors on Amphibian Metamorphosis Assay (AMA) Results**

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The *in vivo* Amphibian Metamorphosis Assay (AMA) has become a key experimental tool for determining the potential of a tested chemical to interact with the hypothalamic-pituitary-thyroid axis of amphibians and other taxa. The 21-day AMA, which is performed using *Xenopus laevis* tadpoles, features a suite of growth and development endpoints that include Nieuwkoop and Faber (NF) developmental stage, snout to vent length (SVL), hindlimb length (HLL), normalized hindlimb length (normalized by SVL; nHLL) wet body weight (WBW), and thyroid histopathology. According to the current wisdom, WBW and SVL are considered to be growth-related types of endpoints, while NF stage, nHLL, and thyroid histopathology are linked more closely to metamorphic development, which is known to be thyroid hormone dependent. Previous AMA studies have demonstrated that experimental exposure of *X. laevis* tadpoles to a thyroid hormone agonist tends to cause increases in NF stage and HLL, decreases or no change in growth endpoints, and decreased histopathologic evidence of thyroid activity. On the other hand, thyroid hormone antagonist exposure generally causes the opposite effects instead. Periodically, however, the results of AMA studies indicate effects which do not line up according to either of these patterns, and the reasons for such incongruous outcomes are often not immediately apparent. The goal of the current presentation is to describe the findings of three recent AMA-type experiments performed independently, that together shed light on ways in which non-endocrine, non-thyroidal factors can influence the AMA study results. This is a cautionary tale that also illustrates the importance of assessing all available endpoints when conducting AMA studies, and the use of a weight of evidence approach for data interpretation.

#### **6.03.P-We385 Development of a Set of AOP Network-Supported IATAs for Thyroid Hormone System Disruption**

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The Partnership for the Assessment of Risks from Chemicals (PARC) is a research and innovation programme funded by Horizon Europe, bringing together nearly 200 partners from 28 countries as well as EU agencies to support next-generation chemical risk assessment and risk management incorporating both human health and the environment in a "One Health" approach.

One of the activities in this context is the development of integrated approaches for testing and assessment (IATAs) for endocrine disruption (Task 6.1.1). The PARC endocrine disruption IATA development process is closely aligned with work on adverse outcome pathway (AOP) development for endocrine and metabolic disruption (Task 5.3.2) to ensure that the methods that are considered for inclusion in the IATAs are supported by key event descriptions of well-developed AOPs.

Thyroid hormone system disruption (THSD) has been identified as one of the priority areas for IATA and AOP development in PARC. Effects on the hypothalamic-pituitary-thyroid axis are considered low-hanging fruit because of the relatively large number of AOPs available or under development for this system, and the ongoing efforts to assess and validate *in vitro* assays for the assessment of THSD conducted at the JRC, the OECD Thyroid Disruption Methods Expert Group, the US Environmental Protection Agency, and within the EURION project cluster under the Horizon 2020 programme.

We present an update on the joint work of developing AOPs and IATAs for THSD in Tasks 5.3.2 and 6.1.1 of PARC. An existing cross-species AOP network for THSD consisting of 50 AOPs taken from the AOP-Wiki, initiated by 13 molecular initiating events and leading to 9 adverse outcomes, provided a starting point. Data and knowledge gaps were identified based on this inventory of available AOPs. Pivotal key events relevant to THSD were identified, and the associated methods were evaluated in terms of data interpretation, available prediction models, test method readiness, and performance. These methods were then mapped to the AOP network, and priorities for further AOP development were determined as a function of IATA development priorities. A set of conceptual IATAs was subsequently outlined, addressing adverse effects that are relevant to either human or environmental health. These IATAs will be further developed and refined in the future in close collaboration with relevant stakeholders, and evaluated through dedicated case studies.

### **6.03.P-We386 Conceptual Framework for Assessing Metal Interactions with the Endocrine System of Aquatic Organisms**

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Regulatory attention on endocrine disrupting chemicals has increased in recent years leading to the ongoing development of criteria and guidance in the European Union to assess the endocrine disrupting (ED) potential of chemicals. These documents, and those developed in other regulatory jurisdictions or in the scientific literature, are largely focused on the ED potential for organic compounds. The applicability of this guidance to metals and inorganic substances is unclear. The available information on the ED properties of metals and metal compounds (hereafter referred to as metals) mainly originates from non-guideline studies in the scientific literature with some conflicting information. Certain studies suggest that some metals may exhibit ED activity in animal experiments intended to evaluate environmental risk. Consequently, concerns have been raised that ED metals may be considered as a new class of hormone-active substances. Some of these assessments, however, are based on a misunderstanding of what constitutes endocrine disruption under current regulatory frameworks. Additionally, some of the experimental evidence stems from studies at exposure concentrations in excess of those that elicit toxicity clearly not related to putative endocrine mediated effects. Although certain metals can cause reproductive and developmental effects, the question remains whether metals specifically and adversely affect endocrine systems at environmentally or physiologically relevant concentrations. Furthermore, their natural occurrence and for some metals their essentiality, including direct roles supporting normal endocrine function, further increases the complexities in the ED assessment of metals. The objective of this presentation is to propose an overall framework for assessing metal interactions with the endocrine system of aquatic organisms. More specifically, we will highlight key issues associated with ED assessment of metals, we will synthesize the available knowledge, identify data gaps, and discuss how this knowledge could facilitate a meaningful ED assessment of metals.

### **6.03.P-We387 Potential Information Requirements for Endocrine Disruption Assessment Under REACH: The Reliance on Animal Data**

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Assessment of substances for Endocrine Disruption (ED) is being introduced in the EU as a specific information requirement under the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) regulation. An independent review estimated potential resource and animal use of two policy options proposed by the European Commission for assessing ED under EU REACH. Here, we discuss potential challenges of implementing the proposed strategies and the balance between *in vivo* testing and other approaches. Further updates to the strategy for ED assessments made prior to this SETAC conference will be taken into account.

The policy options set out the information requirements including potential ways to waive certain studies for ED assessments, though these depend on the availability of higher tier *in vivo* testing or non-specified data showing pathway activity. Many substances would not have sufficient existing higher tier data to fulfil the information requirements, particularly for environmental

assessments. There are also concerns that non-*in vivo* methods (or New Approach Methodologies - NAMs, e.g., quantitative structure activity relationships [QSARs], *in vitro* assays) have not been sufficiently validated or are unlikely to be applicable to industrial chemicals.

The policy options specify data from *in vitro* NAMs under Annex VII information requirements (substances manufactured or imported at >1 tonne) as a trigger for *in vivo* testing and to inform on mode of action analysis. There is concern that the high number of (currently unvalidated) assays will lead to equivocal results, interpretation issues and potentially unnecessary animal testing. Also, *in vitro* test results are largely disregarded at Annex VIII (10-100 tonnes) or IX (100-1000 tonnes), which have direct *in vivo* information requirements, including ecotoxicology endocrine test guideline studies in fish and amphibians. These studies are highly complex, resource and animal intensive. The policy options show little scope for implementing NAMs that might obviate animal testing, such as mammalian *in vitro* assays informing on non-target vertebrates, or reducing the number of animals where possible. The estimated animal use based on either of the policy options is considerable and arguably unachievable considering current laboratory capability and capacity. Further work is required to ensure robust evaluations whilst considering the scale of new animal testing for ED assessments under EU REACH.

#### **6.03.P-We388 Control Performance of Medaka Extended One Generation Test Designs**

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Evaluation of endocrine activity and disruption in human and wildlife involves conduct of specific assays used to evaluate relevant pathways. Multiple *in vivo* test guidelines (TGs) have been validated for mammals, amphibians, or fish, focusing on the estrogen, androgen, thyroid, and steroidogenesis (EATS) pathways. They are now being conducted widely to satisfy regulatory requirements to identify chemicals that have the potential to interact with endocrine pathways. However, these assays are often costly and require the use of a substantial number of laboratory animals, and their outcome can lead to significant regulatory actions. It is therefore critical that the assays are sufficiently reliable and robust.

The Medaka Extended One Generation Test (MEOGRT; OECD TG 240/EPA OCSPP 890.2200) is one such *in vivo* assay designed to provide comprehensive data on adverse effects and endocrine-relevant endpoints for key aspects of the fish life cycle. Exposure starts with spawning fish (F0), covers multiple life stages of their progeny (F1) and continues until hatching (two weeks post-fertilisation) in the second generation (F2). Potential adverse effects on population-relevant parameters are assessed including survival, growth, sex ratio and reproduction (fecundity/fertility). In addition, to provide mechanistic information and linkage between results from other studies where there is evidence for a chemical having the potential to interact with endocrine pathways, liver vitellogenin mRNA (or protein) is measured, phenotypic secondary sex characteristics (SSC) are assessed as related to genetic sex, and gonad histopathology is undertaken.

This poster presents control data from 28 studies conducted in line with the MEOGRT Test Guideline (TG 240/OCSPP 890.2200) or in the spirit of the MEOGRT, including 14 Medaka Multigeneration Tests (MMT) conducted prior to adoption of TG 240 (which includes those used to validate the TG). Reliable historical control data ranges will be developed for the core study endpoints and the associated validity and performance criteria. Additionally, the historical control data will be explored to investigate cross-laboratory and study differences, potential sources of variability, and the power of the test design. The overarching aim is to form a knowledge base that could be used to improve test design, performance, and interpretation of the data. *This abstract does not necessarily represent US EPA policy.*

#### **6.03.P-We389 Distinguishing Non-endocrine from Suspected Endocrine Responses: Importance of Experimental Design**

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Experimental design in ecotoxicological endocrine disruption (ED) studies is vitally important to discriminate between direct endocrine-related effects and effects from other mechanisms of action that may involve indirect compensatory endocrine responses. Further, understanding mechanisms of action is critical to properly classifying a substance as having or not having potential ED properties. ED classification has serious regulatory consequences including banning of substances in some regulatory frameworks, such as those in the EU. Both fish and amphibians, the effect of non-endocrine factors such as bioenergetics, dietary iodine availability, systemic toxicosis, oxidative stress, and treatment-induced stress must be considered in the study design. In addition, the design of amphibian-based studies should be sufficient to detect divergence in developmental rates, as this provides a line of evidence regarding the role of the HPT axis. As an example, we will discuss how careful experimental design of an amphibian test provided support for the hypothesis that the effects of Cu on development and metamorphosis in *X. laevis* are suspected to be the result of non-endocrine systemic toxicity occurring in early development prior to hypothalamic-pituitary-thyroid (HPT)-driven metamorphosis. In his case, additional histopathological assessment beyond that required by standard OECD protocols allows for a better discrimination between direct and indirect related ED effects in the test organisms.

#### **6.03.P-We390 The Importance of Integrating all of the Evidence to Identify Endocrine Disruption: A Case Study with Bisphenol A**

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The challenge of identifying endocrine-disrupting chemicals (EDC) has been at the forefront of regulatory discussion and debate

in the European Union and internationally since the late 1990's. According to the World Health Organization's International Programme on Chemical Safety, an EDC must (1) alter the function of the endocrine system; and (2) as a consequence of that alteration, cause an adverse health effect in humans or wildlife. This definition requires a causal link between the endocrine mode of action (MoA) and the adverse effect that can often be difficult to discern when several potential pathways converge on the same adverse effect. While expanding sets of assays and tools are being developed that provide new and targeted information to better differentiate between endocrine and non-endocrine patterns of responses, the use of this type of data in regulatory decision making is often inconsistently employed. The extensive database for bisphenol A (BPA) provides an excellent case study to illustrate the use of some of these new approach methods for linking mechanistic information to apical outcomes of relevance in a hypothesis testing weight of evidence (WoE) framework. The WoE evaluation for BPA included the integration of data from high throughput screens, regulatory guideline studies and published literature, corresponding to levels 1-5 of the OECD conceptual framework. While BPA exhibits weak estrogenic activity in *in vitro* receptor binding studies and can increase male VTG in fish, the profile of adverse effects that have been measured in BPA-exposed aquatic vertebrates and are used in ecological risk assessments (*i.e.*, effects on survival, growth, development, and reproduction) are not all definitively linked to a weak estrogenic mode of action. The use of bioactivity screens and a consideration of the mechanistic potency of BPA, in relation to endogenous hormones, suggests that the lead mode of action is related to non-specific, systemic toxicity rather than an endocrine specific mode of action.

#### **6.03.P-We391 Efficacy of Dietary Administration in the Amphibian Metamorphosis Assay: Influence of Solvent Loaded Test Substance on Nutritional Quality and Control Performance Criteria**

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Based on a recent regulatory request to perform an OECD TG 231 Amphibian Metamorphosis Assay (AMA) of bis(2-propylheptyl) phthalate (DHPH) administered via diet, an investigation of the efficacy of dietary administration of test substance was performed. The efficacy study was comprised of two phases: evaluation of the physical influence of the loading process via solvent and 10, 1, and 0.1 mg/L test substance or surrogate (sunflower oil) on the Sera Micron Nature® (SMN) diet, and 2) performance of a modified AMA in which Nieuwkoop and Faber (NF) stage 51 *Xenopus laevis* larvae were exposed to dechlorinated tap water using one concentration of the sunflower oil in the diet for 21-days. Sunflower oil served as a surrogate with similar physicochemical characteristics to DHPH. Based on the results of phase 1, the addition of acetone or acetone with DHPH or sunflower oil and subsequent solvent removal physically altered the diet. Specifically, reduced transmittance, density and dietary pellet were observed indicative of alteration of the nutritional quality of the diet. Since physical impact was identified in the acetone treated diet and magnified with the addition of the DHPH or sunflower oil, treatments used in the modified AMA were acetone alone and 0.1 mg/L sunflower oil dissolved in acetone. These treatments were evaluated against an SMN benchmark using standard AMA endpoints. Both the acetone treated SMN and 0.1 mg/L sunflower oil treated diets had significantly reduced survival rates, 67% and 70% relative to the SMN benchmark (100%). Clinical signs of gastrointestinal impaction were noted in the dietary treatments, but not the SMN benchmark. Increased occurrence of tail flexure was also observed in the acetone treated diets (29-38%) compared to the SMN benchmark. Treatment of the MN with acetone (NF stage 58.3) or 0.1 mg/L sunflower oil dissolved in acetone (NF stage 58) decreased the median developmental stage relative to the SMN benchmark (NF 60). Development stage frequency distribution was also decreased relative to the SMN benchmark. Aside from the adversity identified by the present evaluation, the inability to determine doses administered and route of exposure, and lack of historical control data to support this design compound the inefficacy. Ultimately, dietary exposure of a test substance in an AMA study will not provide meaningful data that can be appropriately interpreted and warrants traditional aqueous exposure.

#### **6.03.P-We392 Historical Control Histopathology Data from Amphibian Metamorphosis (AMA) and Fish Short Term Reproduction (FSTRA) Assays**

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The Amphibian Metamorphosis Assay (AMA) and the Fish Short Term Reproduction Assay (FSTRA) are *in vivo* ecotoxicological bioassays that are used to determine if exposure to a tested chemical might have the potential to interact with the endocrine systems of aquatic vertebrates. Specifically, the AMA is designed to investigate potential effects of a chemical on the hypothalamic-pituitary-thyroid (HPT) axis, while the FSTRA investigates effects on the hypothalamic-pituitary-gonadal axis (HPG). Translational extrapolation of effects to wildlife and humans is facilitated by the highly conserved nature of endocrine physiological mechanisms among the vertebrate taxa, and results of these screening assays can help to determine if a substance does or does not appear to display endocrine activity, which in the former case can then be investigated by more definitive testing. It's important to recognize that apparent effects in these assays are not always attributable to endocrine perturbation, but instead may be consequences of treatment-induced stress or non-endocrine toxic responses. Differentiating endocrine from non-endocrine effects can be challenging, but answers may be provided by careful examination of data from various AMA or FSTRA endpoints. As a decision-making tool, HCD are most fairly and effectively used in combination with other criteria in a weight of evidence approach, rather than merely as a gatekeeper to exclude undesirable data points or nullify clear treatment-related effects. A major goal of this endeavor is to present histopathology HCD from a sizeable number of guideline-adherent AMA and FSTRA studies, so that such data become publicly available and can thereby be used to help interpret future study outcomes. A second goal is to perform a granular exploration of the AMA and FSTRA control data in order to identify background lesions, assess the utility of particular diagnostic findings for distinguishing endocrine from non-endocrine effects, and help determine if

specific improvements to the guidance and interpretation procedures may be warranted. This information will be used to inform suggestions for the interpretation of histopathology data from AMA and FSTRA studies.

#### **6.03.P-We393 Evaluation of Yeast-based Reporter Gene Assays Using Thyroid Hormone Receptors of *Homo sapiens* and *Xenopus tropicalis***

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Thyroid hormones are a critical role in cardiac function and brain development, and the act of thyroid hormones is mediated through the activation of nuclear thyroid hormone receptors (TRs). Although in vitro reporter gene assays are widely used to detect endocrine disruptors, a TR-responsive reporter gene assay has not been established except for a few human cell-based bioassays. In the previous study, we developed yeast-based TR responsive reporter gene assays. In this study, we investigated whether thyroid hormones and anti-thyroid hormone compounds were effectively detectable using four yeast-based reporter gene assays for TR $\alpha$  and TR $\beta$  in human and *Xenopus tropicalis*.

In 96-wells plates, yeasts were incubated with the various concentrations of test substances for 16 hours. The antagonistic activities of chemicals were examined by competition between the chemical and thyroid hormone simultaneously added at the various concentrations. To distinguish antagonist activity from the cytotoxicity, the yeast strains established in W303a and corresponding mutants for each receptor that constitutively expressed the lacZ reporter were used for comparisons.

In both of TR $\alpha$  and TR $\beta$  assays in human and *Xenopus tropicalis*,  $\beta$ -galactosidase activity was induced by thyroid hormones in a dose-dependent manner. All anti-thyroid hormone compounds tested in this study inhibit thyroid hormone-induced in human TR assays, but some compounds had no antagonistic effects in the assays for *Xenopus tropicalis*. Further studies are needed to determine whether other compounds can be evaluated in our assays.

#### **6.03.P-We394 Influence of Storage Method, Storage Duration, and Freeze-Thaw Cycles on Measured Plasma Vitellogenin Concentrations for Fathead Minnows**

**Julie Krzykwa, and Joseph Marini, Smithers**

Plasma vitellogenin (VTG) concentration is one of the primary apical endpoints of regulatory test methods using fish models where estrogen/androgen/steroidogenesis modalities are evaluated (e.g., OECD 229/230/234 and OCSPP 890.1350). As VTG is relied upon as a biomarker of exposure to endocrine active compounds, there has been a focus on the high variation observed in reported VTG levels, in the impacts this variation may have regarding assay robustness/animal usage/repeat testing, and how the methodology utilized by different labs to assess VTG concentrations may be a factor in the observed variation. One potential source of variation is sample storage. It is currently unclear how storage methodology and storage duration influence the stability and subsequent analysis of fathead minnow plasma VTG. While relevant guidelines outline a storage procedure for plasma samples (i.e., store with a protease inhibitor at -80 °C), not all laboratories are using the described procedure. In addition, these guidelines provide no clear indication of the stability of samples under this storage regime. The paucity of information regarding the effects of storage method and duration makes it difficult to determine if these factors may be influencing the previously noted variation in VTG concentrations. Therefore, the objective of this project was to evaluate the impacts of storage method, storage duration, and freeze-thaw cycles on plasma vitellogenin concentrations. To achieve this objective, composite plasma samples from male and female fathead minnows were stored under multiple storage scenarios (i.e., with/without a protease inhibitor at -20°C or -80°C) and sample VTG concentrations were measured via ELISA at 0.14 (1 day), 2, 6, and 15 weeks after collection for VTG concentration. The results of this project indicate that storage time and method have an impact on measured plasma VTG concentrations and may be a contributing factor to the high inter- and intra-laboratory variation in control VTG concentrations. In addition, this dataset confirms that freeze-thaw cycles should be avoided if possible; however, if freeze-thaw cycles are anticipated in advance samples should be stored neat.

#### **6.03.P-We395 Thyroid Hormone System-disrupting Effects Of Carbamazepine on Zebrafish – A Case Study for the Performance Of OECD TG 210 (FELS) with Implementation of Thyroid-related Endpoints**

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In recent years, research on endocrine disruptors (EDs) has picked up speed again, since the focus has now included chemicals with disrupting effects on the fish thyroid hormone (TH) system. The hypothalamus-pituitary-thyroid (HPT) axis is crucial for the maintenance of homeostatic functions and numerous developmental processes. Its role can be studied in models such as zebrafish, where changes of TH levels may lead to changes in eye and swim bladder development. As the HPT axis processes are highly conserved among vertebrate classes, cross-species extrapolation is being considered to comply with the 3R principles of replacement, reduction and refinement of animals used for experimental purposes. For environmental screening, current test guidelines (TGs) for thyroid-active compounds are restricted to protocols with amphibians, although fish represent the most frequently used model for ED testing. Nevertheless, the thyroid modality is not covered in existing fish TGs. The zebrafish (*Danio rerio*) is a well-established model in ED testing and is currently being evaluated as a model for the assessment of thyroid hormone system-disrupting chemicals (THSDCs). Various thyroid-related parameters have been identified as relevant endpoints so far: thyroid follicle histopathology, eye development, swim bladder inflation and TH level measurement. The ERGO project, as part of the EU H2020 EURION cluster, is focused on optimizing existing fish TGs for screening for the effects of THSDCs, such as the fish early-life stage toxicity test (FELS, OECD TG 210). For the present case study, zebrafish embryos were exposed during the first 33 days post-fertilization to carbamazepine, an anti-convulsant drug that has been shown to accelerate liver

metabolism and thereby increase TH excretion, lowering TH levels in mammals. In order to compensate the lack of adequate hormone level, due to higher liver clearance of the hormone, thyroid follicles are expected to proliferate. Consequently, to confirm this effect at the morphological level, juveniles were subjected to histopathological analysis of thyroid follicle morphology and epithelium thickness. Moreover, eye development, which is also under the control of THs, was investigated by measuring different retinal layers. These analyses provide further information on the effects of carbamazepine on the HPT axis in fish and on the sensibility of thyroid follicles and eye development as endpoints for THSDCs screening using OECD TG 210.

### **6.03.P-We396 The Rapid Estrogen ACTivity In Vivo (REACTIV) Assay and the Rapid Androgen Disruption Activity Reporter (RADAR) Assay**

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In June 2022 a novel level three OECD test guideline was published for the Rapid Androgen Disruption Activity Reporter (RADAR) assay, TG 251. It describes a 72 h *in vivo* assay for the detection of androgen axis active chemicals using transgenic medaka (*Oryzias latipes*) eleutheroembryos, non-compliant with the EU definition of a laboratory animal.

A complementary assay for the detection of alterations in estrogen axis signalling is currently under OECD validation. The Rapid Estrogen ACTivity *In Vivo* (REACTIV) assay is based on the same concept as the RADAR assay, the use of transgenic medaka eleutheroembryos that reveal the level of activity of the hormonal axis of interest by the expression of GFP. In the case of the REACTIV assay, the axis of interest is the estrogen axis and its activity is translated into fluorescence using a portion of the *choriogenin h* gene promoter to drive the expression of GFP coding sequence under the control of liganded estrogen receptors. The use of aquatic eleutheroembryos allows quantification of the effects of test chemicals acting via a range of modes of action (MoA). The chosen reporter, the choriogenin h promoter, as a terminal step in estrogen axis signalling, indicates the net effect of disruption of the estrogen axis via one or multiple mechanisms.

Laboratories from six countries took part in the REACTIV assay interlaboratory validation exercise. The chosen test chemicals covered a wide range of MoA. The transferability of the assay and its ability to identify chemicals acting on estrogen axis signalling, either at the receptor level or on downstream steroidogenesis was demonstrated. The six expected inert chemicals were all correctly identified as inactive on the estrogen axis. The results obtained by the lead laboratory for chemicals tested with the REACTIV and RADAR assays during the OECD validation exercises showed that all ten chemicals were correctly identified as active or inactive. Chemicals with different MoA showed differing response profiles in the two assays.

The REACTIV assay is a 24 h assay that allows the quantification of estrogen axis activity. It provides crucial data on endocrine activity for a weight of evidence approach. By comparing the results in the presence and absence of testosterone the REACTIV assay can help to elucidate the MoA of a test chemical. A greater precision for identifying the MoA is achieved when the REACTIV assay is performed in parallel with the complimentary RADAR assay.

### **6.03.P-We397 Lessons Learnt from Three Years of Applying the XETA Test Guideline**

**David Du Pasquier and Gregory F. Lemkine, Watchfrog S.A., France**

The Organization for Economic Cooperation and Development has set up a conceptual framework for the testing and assessment of endocrine disrupters (EDs). Different *in vitro* and *in vivo* standardized methods (test guidelines, TG) are categorized into five levels of increasing biological complexity. Detection of thyroid active molecules is addressed by mammalian and amphibian testing. The mammalian testing strategy mainly relies on measurements of plasma concentrations of thyroid hormones (THs) as well as on histopathological analysis of the thyroid. Amphibian testing takes advantage of the THs' exclusive control of anuran metamorphosis. In June 2019, the OECD validated the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) in the test guideline program in order to support the identification of thyroid active chemicals. The XETA utilizes *X. laevis* eleutheroembryos to detect modulation of thyroid signalling by thyroid active chemicals. The assay is transcription-based and uses a transgenic tadpole line expressing the Green Fluorescent Protein (GFP) under the control of a promoter directly regulated by TH. The response measured is fluorescence of embryos. The technical transfer of the assay to contract research organisations (CROs) was a prerequisite to the application of the XETA, the test guideline has been easily implemented in several CROs in a short time frame. The use of the XETA has been supported by the publication of an annex describing how to include the XETA in the assessment strategy of the ECHA/EFSA Guidance. To date reglementary studies have been performed to assess the T-modality for approximately 30 chemicals using the XETA. During these studies, some practical points of consideration have appeared including how to consider sub lethal effects, affecting behaviour or obviously testifying of toxicity, in the definition of the Maximum Tolerated Concentration and how to ensure testing concentrations close as possible to the MTC. The objective of the present communication is to highlight these developments that allowed the use of the XETA in chemical assessment since its validation in 2019. The development and rapid implementation of the XETA into the reglementary assessment of chemicals has paved the way to developing assays using embryonic stages of aquatic vertebrates. These are ethical and novative tools that perfectly fits into the strategy of a mechanistic approach for the comprehensive assessment of endocrine activity on targeted EATS.

### **6.03.P-We398 Elucidating the Effects of Acute and Chronic Exposure of 2,4-dichlorophenoxyacetic Acid on Freshwater Fish Innate Immunity**

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Aquatic herbicides, such as 2,4-Dichlorophenoxyacetic acid (2,4-D) formulations, are commonly used on invasive species for

management throughout the United States. Ecologically relevant concentrations of 2,4-D impair essential behaviors such as locomotion and prey capture, reduce survival, and act as an endocrine disruptor; however, there is limited knowledge of its effects on the health of non-target organisms. Here, we aim to broadly investigate the acute and chronic exposure impacts of 2,4-D on condition, immune function, and fever-avoidance behaviors of fathead minnows (*Pimephales promelas*), a model organism. To elucidate the effects of 2,4-D, we took a multi-faceted approach. First, we exposed adult male and female fathead minnows to three different ecologically relevant concentrations of 2,4-D (0.0, 0.4, and 4.0 mg/L) and took blood samples at three acute time points (6, 24, 48, and 96 hours) and one chronic time point (31 days). Male fatheads had significantly increased mean total white blood cell counts after 6-hours of exposure at 0.4 and 4.0 mg/L compared to control fish; however, individual cell types did not change with treatment. Female fatheads exposed at 4.0 mg/L had increased heterophils at a chronic timescale, but no other significant changes were observed. Second, we are assessing the ability of male fathead minnows to have a “healthy” behavioral fever response after exposure to lipopolysaccharide (LPS) and when exposed to 2,4-D. We expect that control fish would prefer warmer water environments during a stimulated fever response to naturally raise their body temperatures to clear an infection. However, we expect fish exposed to 2,4-D will not be able to make the same behavioral choices due to neurological impairment as compared to the control fish. Finally, we are exploring innate immunity-associated gene expression changes after chronic exposure to 2,4-D throughout development. Results from these experiments will shed light on freshwater fishes ability to maintain an adequate immune system and clear pathogen infections while exposed to commonly used herbicides. Moving forward, our group will build on these preliminary studies with an ultimate goal of elucidating the morbidity impacts of herbicide exposure on fishes health and immunity. These studies will help management agencies accurately make data driven management practices and create risk assessments for herbicide treatments in aquatic ecosystems in the Great Lakes, and beyond.

#### **6.03.P-We399 Testing for Endocrine Disruption - Existing Gaps and Recommendations from a CRO Perspective**

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Testing for endocrine disruption is an essential part of regulating chemical substances in the EU and USA. Accordingly, contract research organisations (CROs) face a high demand for endocrine testing. Since 2019, IES Ltd. has been increasingly conducting key *in vivo* bioassays out of Level 3 or 4 of the OECD conceptual framework for testing and assessing endocrine disruptors, such as the Fish Short Term Reproduction Assay (FSTRA; OECD 229, OPPTS 890.1350), the Amphibian Metamorphosis Assay (AMA; OECD 231, OPPTS 890.110) or the Fish Sexual Development Test (FSDT; OECD 234). Especially for the registration of plant protection products (PPPs) in Europe, the FSTRA and AMA represent critical assays to screen for endocrine disruption given that the indication of an effect triggers higher tier tests resulting in additional costs and use of animals and, in case of a confirmed endocrine disrupting effect, a ban of the substance on the European market. Based on our long-term experience and during the implementation of the FSTRA and AMA, we identified gaps in the description of the applied test procedures in both the OECD and OPPTS guidelines, which may affect the reproducibility and comparability of the outcomes. Given the high importance of these tests for the registration and re-registration of PPPs, existing gaps should be discussed and closed for future testing. Thus, to identify weaknesses of the current procedures, we compared our control data for all relevant endpoints (fecundity, nuptial tubercle score, vitellogenin for the FSTRA and determination of the Nieuwkoop-Farber stage (NF-stage), measurement of the hind limb and snout-vent length for the AMA) with control data from the EPA Endocrine Disruptor Screening Program. Based on this comparison, we will discuss critical steps of these key bioassays to screen for endocrine disruption and provide recommendations to improve the testing procedure, such as choosing suitable test concentrations due to the lack of reliable toxicity data.

#### **6.03.P-We400 Investigation of Possible Endocrine Sensitive Endpoints in Snail Embryos and Juveniles**

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Today, test methods and endpoints able to identify chemicals interfering with the endocrine system and thereby disrupt fundamental processes such as development, reproduction, and metabolism, in invertebrates are lacking. Invertebrates, accounting for 95% of all living animal species, are suffering from rapid global decline; 50% of freshwater mollusk species are threatened with extinction. Chemical pollution, including endocrine disrupting chemicals (EDCs), is considered as a key driver, but understanding the scale of the problem is hampered by lack of knowledge; there are very few data available on fundamental endocrinology and neuroendocrinology including regulation of reproduction, development and metabolism of most invertebrate groups. Invertebrates have also received increasing attention in regulatory chemical testing. The 3Rs concept involves replacing vertebrate animal experiments when possible, Reducing the number of vertebrate animals used, and Refining experiments to minimize the impact on vertebrate animals by developing new approach methodologies (NAMs) including less sentient species (e.g. invertebrates) or life-stages (embryonic life-stages).

The great pond snail (*Lymnaea stagnalis*) is a freshwater gastropod mollusc widely distributed in Europe, and it is used as test organism in the OECD test guideline 243, which is a reproduction test designed to assess effects of exposure to chemicals on the reproduction and survival. Currently, we are developing an assay with *L. stagnalis* embryos and juveniles and investigating potential endocrine sensitive endpoints.

Here, we discuss the lack of knowledge about fundamental endocrinology molluscs and present our preliminary experiences and data.

This knowledge is highly necessary for the optimization of existing and/or development of new OECD test guidelines, and for understanding better the potential harmful effects of chemicals on invertebrate populations in the environment.

### 6.03.P-We401 Underlying Mechanisms of Drug-Induced Endocrine Disruption to Gastropod Shell to Be Unravelling by Fundamental Molecular Diagnostics

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Due to a lack of detailed knowledge relating to the endocrinology of molluscs, current OECD tests lack mechanistic underpinning. Therefore, current freshwater gastropod OECD tests (242 and 243) can only determine reproductive toxicity in adult snails. Consequently, existing OECD tests are unable to identify if a chemical is an endocrine disruptor (ED) to molluscs. In the last decade, molecular and chemical exposure investigations have shown that molluscs do not possess a nuclear androgen receptor, and do not respond to exogenous androgen (e.g. testosterone) exposure. However, molluscs do have some steroidogenic genes which are homologous to vertebrates. These include 5- $\alpha$  reductases (5 $\alpha$ R) known to convert testosterone to the more potent dihydrotestosterone in vertebrates. As yet, the function of these steroidogenic genes in molluscs is unknown. Previous work in our lab has shown that 5 $\alpha$ R genes are not only expressed in adult and embryonic *Biomphalaria glabrata* tissues, but that developmental exposure to pharmaceutical 5 $\alpha$ R disruptors (dutasteride or finasteride) results in a highly reproducible and dose-dependent disruption to embryonic shell morphology. Here we present further investigations narrowing down the sensitive window of dutasteride disruption in developing *B. glabrata*. To further understand this developmental disruption, we also need to map gene expression profiles in the developing embryos. However, basic molecular information, such as suitable endogenous control “housekeeping” genes during embryo development are still lacking in this species. Candidate endogenous control genes and primers were identified from the literature. Their expression stability across four stages of embryo development (days 2-5 postoviposition) and albumen gland tissues has been investigated using RT-qPCR and the stability algorithm BestKeeper. Preliminary analysis suggests Ubiquitin as the most stable endogenous control gene out of the candidates tested. Optimisation and refinement of primers of the most appropriate control genes are being re-validated. The development of robust endogenous control genes will allow us to link 5 $\alpha$ R gene expression patterns to the sensitive window of dutasteride disruption, furthering our understanding of this possible ED sensitive phenotype in gastropods. These control genes will enable expression analysis of other transcripts during gastropod early development for mechanistic research and could inform future ED test development.

### 6.03.P-We402 Assessment of Sediment Samples from Lake Tai, China via Effect-Based Methods Including Metabolic Activation

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The quality of ecological environment of Lake Tai, the third-largest freshwater lake in China, is facing huge challenges due to existing input of chemical pollution since last few decades. Among numerous emerging pollutants, endocrine disruptive chemicals (EDCs) with the ability to disrupt the mammalian hormonal system attracts more attention. Different methods have been used to detect those compounds. As a supplementation of chemical target analysis, *in vitro* chemically activated luciferase gene expression (CALUX<sup>®</sup>) was proposed as a standard testing for endocrine disruptors in Europe. However, the currently used cell-based CALUX<sup>®</sup> assay may not provide full potential of adverse effects of environmental samples because of its limited metabolization capacity. We standardise the testing system by adding the rat liver S9 mix as an exogenous metabolic system to the *in vitro* CALUX<sup>®</sup> assay for (anti-)estrogenic (ER $\alpha$ ) and (anti-)androgenic receptor (AR) assessment as the first step. To exploring comprehensive toxicological potential of Lake Tai, sediment samples from three sampling sites were taken and subsequently tested with *in vitro* CALUX<sup>®</sup> assay without and with S9 metabolic module. By the neutral red retention assay, 0.01 protein mg/ml of S9 mix was more suitable as working concentration compared to 0.05 protein mg/ml, 0.1 protein mg/ml. Furthermore, *in vitro* CALUX<sup>®</sup> assay combined with S9 metabolization were completed systematically with tamoxifen, flutamide and other new chemicals of benzo(a)pyrene as the agonist in the ER $\alpha$  CALUX<sup>®</sup> assay and stanozolol as androgen agonist in the Anti-AR CALUX<sup>®</sup> assay. The establishment and improvement of standardization of *in vitro* CALUX<sup>®</sup> assay with metabolic fraction not only provide more comprehensive ecotoxic evaluation to environmental samples as powerful screening tool, but also implies that the addition of S9 mix could overcome the disadvantage of the lack of metabolic bioactivity of various cell-based mode of action bioassays.

### 6.03.P-We403 Peer-Group Assessment of the Results from an Interlaboratory Study for Effect-based In Vitro Methods to Determine Estrogenic Effects in Environmental Surface Water

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Estrogens are well known for their potential of causing adverse effects on aquatic organisms in extremely low concentrations of exposure (ng to pg per liter) and are already monitored for many decades, accordingly. Hence, environmental quality standards for priority substances under the European Water Framework Directive for 17- $\alpha$ -Ethinylestradiol (EE2), 17- $\beta$ -Estradiol (E2) and Estrone (E1) have been suggested and reviewed. The use of effect-based *in vitro* methods (EBM) for the determination of hormonal effects contributes beneficial information on the assessment of the quality status of environmental water ecosystems especially in the case of estrogenicity. Since the publication of the ISO 19040 standards in 2018 the respective *in vitro* assays are in use for the analysis of estrogenicity of surface water in routine laboratories. The implementation of internal and external quality assurance procedures is an essential requirement for providing confidence and reliability in the reported estrogenicity data by the responsible laboratory. Thereby it still remains challenging to compare and harmonize the generated data due to the use of mammalian and yeast biosensor systems exhibiting different biological responses with respect to the relevant estrogens. To provide an opportunity to implement an external quality assurance measure which also meets the requirements of the ISO/IEC

17025 an interlaboratory study was performed to determine the estrogen equivalent concentration (EEQ) in artificial environmental samples.

Several European laboratories participated in the study by using different effect-based *in vitro* methods. Due to the different biological responses of the *in vitro* methods a combined evaluation was not feasible. However, as the number of participants per method type was too small for a reliable evaluation of laboratory performance, a novel mathematical-statistical approach has been applied that considers peer groups. Using this approach, the peer group effect due to the use of different effect-based *in vitro* methods (ISO 19040-1 and other, ISO 19040-2, ISO 19040-3 and other) as well as the overall robust mean across peer groups were considered. Following this approach, the assessment of laboratory performance as well as the characterization of differences between peer groups (method effects) was obtained.

## 6.04 Deriving and Implementing Ecologically Relevant Environmental Quality Standards

### 6.04.P-Th392 pH normalization of effect values of ionisable drugs for aquatic EQS derivation taking Diclofenac as an example

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Around 80% of prescribed drugs are ionizable. Ionizable substances tend to be more toxic and bioavailable with an increasing fraction of the unionized species. However, aquatic toxicity tests are mostly performed at pH adapted to the test organisms rather than to the requirements of the ionizable substance. We hypothesize that heterogeneity in aquatic toxicity effect data is at least partially caused by differences in bioavailability at different pH. Using the weak acid diclofenac, a widely prescribed anti-inflammatory drug as an example, we examine the influence of pH on published effect data and derivation of environmental quality standards (EQS) for freshwater by applying a logD-based correction to effect values.

Around 50-67% of available effect data had to be excluded from the dataset due to lack of analytical verification and reported pH values. This also led to exclusion of rarely tested species and certain groups of organisms. We therefore recommend pH values and exposure concentrations to be determined and fully reported in toxicity studies.

Amphibians are the most sensitive group in the logD corrected acute dataset but are not represented in the chronic dataset. This data gap should be addressed to make the suggested EQS more robust. Likewise, bivalves are the most sensitive group in the logD corrected chronic dataset but are not represented in the acute dataset. This data gap is, however, less relevant due to the chronic type of exposure to diclofenac and other drug substances in surface waters.

Correction of effect values for logD at pH 6 and pH 8 results in a similar decrease in the standard deviation of log10 acute and chronic effect values in both cases. The wide range of pH values allowed in guidelines and used in toxicity tests apparently results in artificial heterogeneity in toxicity data.

The original threshold value (HC5) derived from a species sensitivity distribution (SSD) based on chronic data and pH-corrected data are similar to the lowest effect concentrations in the chronic dataset. The application of an AF of 5 to the respective HC5 results in an EQS<sub>SSD</sub> of 0.057 µg/L for non-corrected data, 0.004 µg/L for pH6-corrected data, and 0.097 µg/L for pH8-corrected data.

We conclude that the decrease in data variability upon pH/logD correction and the substantial differences between EQS derived for different pH values show that pH/logD correction should be mandatory for ionisable substances for hazard assessment and EQS derivation.

### 6.04.P-Th393 Updating the Environmental Quality Standard for nickel in the UK

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Nickel was identified as a Priority Substance under the Water Framework Directive (WFD) and an Environmental Quality Standard (EQS) was set in 2013 that applies throughout Europe. The EQS was set as a “bioavailable nickel” concentration, and the assessment of compliance against it should take account of bioavailability. The EQS was set based on a Species Sensitivity Distribution (SSD) for nickel that is normalised to the local site-specific water chemistry conditions using the Biotic Ligand Model (BLM). The ecotoxicity database that is used as the basis for the SSD, and the underlying models that define the bioavailability normalisation within the BLM, are based on information that was available in 2010. There have been several advances in the science that supports this approach over the past decade, in terms of additional ecotoxicity data that mean a greater diversity of species could be included, and the applicability of the underlying bioavailability normalisation models to broader ranges of water chemistry conditions. Since the UK left the European Union it is free to make an independent evaluation of the nickel EQS if the EU update is deemed inappropriate. This study describes the update of the approach and the derivation of the revised EQS for the UK, and provides a provisional assessment of compliance with the updated EQS for UK freshwaters.

### 6.04.P-Th394 Metals Ecotoxicity Species Sensitivity: From Native Waters to Laboratory Waters

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An informal meeting was held at the 2022 SETAC North America Annual Meeting in November to consider the following question: are metals ecotoxicity results impacted by sensitivities or stresses introduced when organisms are tested in waters not



resembling their natural environment? This meeting was conducted under the auspices of SETAC's Metals Interest Group (MIG). A total of 28 ecotoxicologists/chemists participated.

There is much interest in determining the sensitivity of organisms (e.g., fish; invertebrates, including snails and mussels; and algae) collected from their natural habitat and transported to a laboratory for subsequent ecotoxicity testing with metals and other substances. This has drawn considerable interest particularly for rarely tested and/or endangered species (e.g., sturgeon).

However, as the number of good quality publications increase in this area of research, concerns arise on their suitability for use in risk assessment, in classification, and on development of specific water quality criteria requirements for metals.

Several critical factors in determining the suitability of these studies were discussed in the meeting including: (1) study design requirements; (2) reproducibility of studies in the laboratory with organisms collected at different times or environments; (3) water quality differences between natural waters and laboratory waters; and (4) performance quality criteria.

It was agreed that further research and evaluation of available data were required and that this could be taken forward to a second meeting to discuss future potential activities such as review of existing studies and design of targeted new studies.

#### **6.04.T-01 Deriving an EQS for Aluminium in UK Surface Waters**

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Aluminium is ubiquitous in the environment, although the levels in surface waters may be enriched by anthropogenic activities that result in emissions from both point and diffuse sources. Aluminium can be present in a wide variety of different forms in aquatic systems ranging from truly dissolved simple inorganic complexes, colloidal complexes with organic matter, inorganic colloids, precipitates, and clay minerals. Several of these forms can contribute to adverse effects on aquatic organisms, either by direct toxicity or by physical effects such as smothering or precipitation on gill membranes. Consequently, deriving an acceptable limit that can be practically applied for regulatory purposes is challenging not only because of the different forms that may cause effects, but also because of the need to be able to identify those forms analytically in a manner that is practical for routine regulation.

A number of (bio)availability models have been developed for aluminium based on semi-mechanistic biotic ligand modelling (BLM) principles and empirical multiple linear regression (MLR) approaches to predict the toxicity of aluminium to a variety of standard laboratory test species. Some data is also available that can be used to evaluate the performance of these models both for other species and for natural, field collected, waters. These validation studies are required by many regulators to demonstrate that the bioavailability models will be protective of real ecosystems in natural waters.

This study describes the evaluation of the available evidence on aluminium toxicity to aquatic organisms to derive an EQS that can be practically implemented by UK authorities in regulating discharges containing aluminium into the aquatic environment.

#### **6.04.T-02 On the Derivation and Implementation of Environmental Quality Standards for Sediments.**

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The Swiss Centre for Applied Ecotoxicology (Ecotox Centre) has derived environmental quality criteria for surface waters for many substances, including pesticides, biocides, pharmaceuticals and industrial chemicals. The derivation is largely based on the EU Technical Guidance for Deriving EQS (TGD for EQS), because the protection goals of the Swiss water protection law and ordinance and the Water Framework Directive (WFD) are comparable. In contrast to the WFD dossiers, sediment and human health via the consumption of fisheries products are not part of the EQS derivation in Switzerland. In the last years, the Ecotox Centre has been working on a sediment quality assessment strategy for Switzerland. The objective is to harmonize and improve practices in sediment quality monitoring and assessment, which mainly addresses the chemical status so far. In this context, sediment quality criteria have been derived for both traditional sediment contaminants and other contaminants including pesticides, pharmaceuticals and personal care product ingredients.

At last SETAC Europe Annual Meeting in Copenhagen, we summarized our experience on the derivation of environmental quality standards for sediments as a poster contribution in the session "Contaminated sediment: an environmental compartment of concern". We would like to take the opportunity of this year's session dedicated to the derivation and implementation of ecologically relevant environmental quality standards to put on the spotlight the derivation and implementation of environmental quality standards for the sediment compartment. Building on our last year's contribution, we would like to highlight the difficulties in the derivation of sediment quality guidelines due to the (lack of) availability of relevant ecotoxicological data and the quality and reliability of available data from spiked-sediment toxicity tests, potential issues with implementation of the derived EQS for sediments, and other topics that still deserve further developments such as the incorporation of bioavailability in compliance check, how to address endocrine potential, or how to derive sediment EQS for protecting against secondary poisoning.

#### **6.04.T-03 The ecological relevance of Environmental Quality Standards for biota**

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Aquatic environmental pollution is a transboundary problem. Therefore, substantiated international environmental standards are imperative. For 11 (groups of) pollutants that tend to strongly biomagnify, mostly due to their hydrophobic or lipophilic characteristics, European Environmental Quality Standards exist (e.g. PFOS, PBDEs, PAHs, mercury, dioxins). These compounds

need to be monitored in biota (EQS<sub>biota</sub>), preferably occupying high trophic levels. Even though large and frequent exceedances of the current standards are reported on a global scale, for multiple compounds, no imminent environmental effects are detected. This led to a doctoral research evaluating the ecological relevance of the current EQS<sub>biota</sub>. Firstly, an extensive 4-year monitoring campaign was performed at 44 sampling locations covering the main water bodies in the northern part of Belgium (Flanders). Study species were European perch (*Perca fluviatilis*), European eel (*Anguilla anguilla*), Dreissenid mussels (*Dreissena sp.*) and Asian clam (*Corbicula fluminea*). This resulted in an overview of the general pollution distribution and extend of the exceedance of the current EQS<sub>biota</sub>. Secondly, the potential human health risk of the calculated concentrations was evaluated. Thirdly, critical accumulated concentrations in biota were determined using biotic indices reflecting the overall water quality of each sampling location. Finally, all three approaches were integrated, which suggested that the current EQS<sub>biota</sub> for Hg and PBDEs might be too low to protect the ecological water quality, while the standard for HBCD may be too high. The results from this study provide an important baseline for the further evaluation of the current EQS<sub>biota</sub>. Furthermore, it was brought to light that bioaccumulated concentrations of PFOS and dioxins in Flanders are very high, potentially causing both environmental and human health risks.

#### **6.04.T-04 Thresholds for the terrestrial environment: proposal for an aggregated Environmental Quality Standard**

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The EU Soil Strategy for 2030 ‘Reaping the benefits of healthy soils for people, food, nature and climate’ envisages that all soils in the EU should be in a healthy condition by 2050. For this, one of the legal instrument would be a possible EU Soil Health Law for protecting, sustainably managing and restoring EU soils. In the preparation of this law, the European Commission included in its consultation process questions related to the use of thresholds: this concerns for example the identification through measurements and monitoring of soil health and threats of early warnings of exceedances of critical thresholds to guide sustainable soil management. The harmonization at EU level of the threshold values for contaminants that would trigger a risk assessment is also questioned.

Currently different values are derived for the purpose of risk assessment including ground organisms, above ground organisms or human health. For the purpose of soil monitoring however, and from the experience gathered with the implementation of the Water Framework Directive, it appears that a single value might be useful and simplify the development of analytical and management activities.

However, whereas the Water Framework Directive has set the scene for the definition of safe level that should not be exceeded in water, soil and biota to protect the human health and the ecosystems, such a definition does not exist for terrestrial environment. Methodological developments would be needed to achieve an equivalent approach for soil. This work is a feasibility study, which reviews the different protection goals that need to be considered and the possible approaches to combine them in a single value. Whereas the Soil Health law remains in preparation, this study does not question the relevance of protection goals that is a policy decision, but rather highlights the feasibility of including the definition of a single aggregated threshold value that could help for the implementation of monitoring requirements in soil, early warning on chemicals of concern and the identification of local exceedance.

Example on data rich and data poor chemical indicates that whereas these differences exist also for aquatic toxicity, they are more marked for terrestrial toxicity endpoints. The definition of an aggregated threshold value is however feasible, but technical adaptations would benefit to be further refined.

#### **6.04.P Deriving and Implementing Ecologically Relevant Environmental Quality Standards**

##### **6.04.P-Th395 Enhancing the Role of Open Literature in Regulatory Environmental Risk Assessment**

**Simone Rizzuto, Maria Arena, Domenica Auteri, Fernando Alvarez, Franco Ferilli, Aude Kienzler, Alessio Ippolito, Alberto Linguadoca, Rachel Sharp, Csaba Szentes and Laura Villamar-Bouza, European Food Safety Authority, Italy**

Regulatory environmental risk assessment (ERA) of chemicals is often targeted by academic researchers and by the general public for relying mainly on industry dossier studies, and for not being sufficiently receptive of literature studies. In the case of plant protection products (PPPs), data requirements include standard GLP studies (e.g., OECD, ISO), as well as all relevant scientific peer-reviewed open literature on the active substance, its relevant metabolites and the representative formulation(s) published within the last 10 years before the date of submission of the dossier. In 2011, EFSA published a guidance document providing instructions on how to minimize bias in the identification, selection and inclusion of peer-reviewed open literature in dossiers, according to the principles of systematic review. Thus, in principle, studies published in the peer-reviewed open literature could directly inform the regulatory ERA of PPPs, mainly in addressing their hazard characterization. One of the pillars of the EU chemical strategy for sustainability is the concept of ‘one substance, one assessment’. In this framework, the assessment of hazard data performed in the context of the environmental risk assessment of PPP for substance/product authorization can be relevant for the selection of Environmental Quality Standard (EQS). Depending on the relevance and the reliability of the measured endpoints, the results from such non-regulatory studies could be used either quantitatively (e.g., when an endpoint addressing a data requirement can be estimated from the study), or qualitatively (e.g., as supporting information in a weight of evidence approach or to further investigate any remaining uncertainties) in the hazard characterization, together with the available dossier studies. Nonetheless, it is currently rather uncommon for the regulatory ERA of PPPs to be driven by endpoints obtained from the open literature.

In this contribution, an analysis of the most frequent issues hindering relevance and reliability of literature studies in regulatory ERA, was conducted over several studies gathered during literature reviews of active substances/formulations. This input aims at: (i) delineating the issues often observed in the relevance and reliability assessment of open literature studies; (ii) offering possible

ways to produce useful outcomes for the regulatory process, while maintaining the freedom to investigate specific and independent research questions.

#### **6.04.P-Th396 A Framework for the Co-building of Reference Values Using Multi-agent Approach Implementing Exposure-impregnation Models**

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Embedding laboratory or mesocosm studies within monitored landscape in order to assess the real impact of stressors at the ecosystem scale is a huge challenge for ecotoxicological modelling. Assessing the ecological risk of chemical compounds is critical to avoid the decline and potential extinction of some populations that may subsequently threaten other species for example in food chains. Simultaneously, being able to control population dynamics is also important when a population outbreak in specific time and space may compromise agricultural activities and human health.

Ecological Risk Assessment studies have to deal with multiple exposure stressors (chemical and micro-biological) within multiple life-history contexts. In this talk, we propose a unified modelling framework for ecotoxicology, agroecology and eco-epidemiology (see Figure 1).

However, although these models are often intended to help decision-making, their use too rarely translates into direct application [1]. The obstacles are distributed between the lack of relevance (i.e., scales and variables of the model not appropriate to the decision-making framework), the low credibility (i.e., not following the standards of scientific rigor) and the lack of legitimacy (i.e., lack of trust of the users in the results). Therefore, we propose a first step of a methodology using social-science to parameterized and questioned the model.

Our results illustrate the robustness of this framework through its application within two contexts: (i) the impact of Bt-maize on Non-Target Lepidoptera in Catalonia (Spain), and (ii) the outbreak of Fall Armyworm resistant population at the continental scale of Australia. We also performed a sensitivity analysis in both cases to identify the most influential processes and provide recommendations of reference values for ecosystem management practices.

#### **6.04.P-Th397 Solution-focused Mixture Assessments at the Landscape Level and Key Notions to Improve Water Quality Under the Water Framework Directive**

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Surface water of good quality is vital for human health, ecosystems and their services, which provides the motivation of the ‘toxic-free environment’ aspiration. However, a low percentage of water bodies in European countries is tagged with having good water quality, with the Netherlands having a score of only 1% at the landscape-level. The main challenge is clear: “how to reach a 100% soon?” and how to avoid the backlash that >20 years of Water Framework Directive (WFD) policies apparently yield high ‘failure’ scores?

This challenge was addressed in the EU-projects *SOLUTIONS* and the Dutch project “*Knowledge Impulse on Water Quality*” by close consideration of field facts on exposures, risks and impacts in comparison to policy principles and operational solutions. Upon close reading of the WFD and the *Chemical Strategy for Sustainability* (CSS), and collecting and analysing monitoring data for Dutch surface waters over a prolonged period, this presentation will suggest some key innovations to forward WFD practices. The key innovations are based on implementing a ‘distance to target’ metric to summarize spatial or temporal trends in chemical pollution of surface waters next to the well-known environmental quality standards, and to provide water managers with two main new tools: (1) the solution-focused assessment paradigm, to help forward thinking into feasible solutions, and (2) a website with tools that assist in using that paradigm to evaluate efficacy of past solutions and explore likely outcomes of novel ones. The innovations were designed together with water quality management practitioners.

Given >80k visitors to the mixture risk website in the first few days upon publication marks an apparent broad public interest. Case studies further show spatial and temporal trends in chemical pollution. Given examples of positive effects of management on water quality and biodiversity, it appears key to utilize “distance to target” (DTT) approaches next to the current “one out all out” (OOAO) approach to report and assess real, gradual, trends in chemical water quality and enable the design of effective measures.

#### **6.04.P-Th398 Criteria to Assess the Relevance and Reliability of Environmental Threshold Concentrations (ETCs) for Pharmaceuticals**

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Environmental Threshold Concentrations (ETCs) are threshold values below which concentrations are ‘unlikely to represent a risk to surface waters’ (European Medicines Agency) and other environmental compartments. ETCs indicate the maximum concentration for which ‘safe use of a chemical’ (REACH) can be assumed, given the available data and the context of the particular legislation. ETCs include PNECs (Predicted No Effect Concentrations), EQSs (Environmental Quality Standards), and other similar terms used in environmental science, risk assessment and risk management. For pharmaceuticals (similar to other substances), multiple ETC values might be available from regulatory frameworks and scientific literature. When performing a risk

assessment, often the question is which of these values should be used, i.e., which is most relevant and reliable given the intended use? In this poster, we present a method to assess the relevance and reliability of ETC values.

#### **6.04.P-Th399 Updating the EQS for Zinc in the UK**

**Adam Peters<sup>1</sup>, Iain A Wilson<sup>1</sup>, Graham Merrington<sup>1</sup> and Christopher Cooper<sup>2</sup>, (1)wca environment, United Kingdom, (2)International Zinc Association, Belgium**

Environmental Quality Standards have been set for zinc in UK waters for several decades. Zinc was identified as a specific pollutant under the Water Framework Directive in the UK and standard updated accordingly. The EQS for zinc was last updated over a decade ago and advances in the understanding both of zinc toxicity to aquatic organisms and the bioavailability modelling that is used to ensure that the EQS applied is ecologically relevant to local conditions mean that it may be appropriate to update the EQS. Updates to the bioavailability modelling approaches used may mean that the standard can be applied over a broader range of water chemistry conditions than was previously possible, meaning that the true risk posed by zinc can be evaluated for more of the surface waters resulting in a more ecologically relevant EQS. The updated standard will be compared against the existing EQS for zinc in UK freshwaters, in terms of the size and taxonomic diversity of the database, the proportion of UK water chemistry conditions covered, and the likely levels of compliance if it is updated. This presentation summarises the updates made and their implications for the EQS for zinc in the UK.

#### **6.04.P-Th400 Updating the Metals Bioavailability Assessment Tool for Assessing EQS Compliance for Bioavailable Metals in the UK**

**Adam Peters<sup>1</sup>, Iain A Wilson<sup>1</sup>, Graham Merrington<sup>1</sup> and Helen Wilkinson<sup>2</sup>, (1)wca environment, United Kingdom, (2)Environment Agency (England), United Kingdom**

Several metals have Environmental Quality Standards set as bioavailable concentrations in the UK. Biotic Ligand Models are usually used for predicting metal toxicity under local water chemistry conditions, but they are data intensive, complex, and required skilled personnel to use them. Consequently, simplified tools are often used by regulators to make compliance assessment more practical and cost effective. The Metals Bioavailability Assessment Tool (MBAT) was developed for UK regulatory authorities to assist in compliance assessments against the EQS for copper, manganese, nickel, and zinc as a simple spreadsheet-based tool that could be easily operated, and if necessary could be implemented directly within automated Laboratory Information Management Systems. This tool therefore needs to be updated if the underlying EQS values for any of the metals are updated, for example to take account of new ecotoxicity information, or to extend the range of water chemistry conditions that the bioavailability approach can be applied over. This presentation describes changes made to the tool, including the approaches used to implement these changes, for possible future updates to metal standards in the UK.

#### **6.04.P-Th401 Which Distribution to Choose for Deriving a Species Sensitivity Distribution?**

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Species sensitivity distributions (SSDs) are used to assess ecological risk and provide critical information to derive defensible guideline values. SSDs are typically estimated by fitting a statistical distribution to ecotoxicity data and several distributions have been used following existing guidelines. However, it has not been quite clear yet which statistical distribution should be chosen for SSD estimation, and how the choice of the distribution affects the hazardous concentration for 5% of the species (HC5). This study aimed to compare three different SSDs (estimated using log-normal, log-logistic, and Burr Type III distributions), based on an information criterion and HC5 values derived from the three SSDs. The ecotoxicity data for 192 chemicals were collected from a database and selected based on the criteria proposed in previous studies. The SSDs were estimated by fitting the three distributions, and a distribution model with the smallest value of the corrected Akaike's information criterion (AICc) was selected as the best model in terms of prediction. HC5 values estimated from sub-optimal models were compared with those estimated from the best models. As a result, out of 192 chemicals, log-normal, log-logistic, and Burr type III distributions were selected as the best models for 99, 61, and 32 chemicals, respectively, based on AICc. The log-normal distribution served as the best model in half of the cases (52%), and this finding provides some support to the fact that the log-normal distribution has been widely used for SSD estimation. The ratios of HC5 values estimated from log-normal distribution to those estimated from log-logistic distribution fell within the range -0.5 to 0.5 (log<sub>10</sub>-transformed values), suggesting the differences between the HC5 values estimated from these two distributions were relatively smaller. In contrast, for example, when the Burr type III distribution was selected as the best model, the ratios of HC5 values estimated from the best model to those estimated from the sub-optimal models fell outside the range -0.5 to 0.5 (log<sub>10</sub>-transformed values) in 18 SSDs. These results suggest that applying only a single distribution for SSD estimation can lead to an under- or overestimation of HC5. More details will be discussed in the presentation.

#### **6.04.P-Th402 Organic Wastes Applied to Land—Australian Approaches to Assessing Risks and Establishing Acceptable Levels**

**Jackie Wright, Environmental Risk Sciences, Australia**

Resource recovery is encouraged by regulators in Australia through zero waste and circular economy policies. Such policies have driven proposals for reuse of a wide range of different organic wastes including biosolids, food and garden organics (FOGO), and organic wastes recovered from general kerbside garbage (i.e. municipal solid waste, MSW).

Pre-existing Australian regulations require all businesses to minimise the risk of harm to the environment from site activities.

Wastes recovered in alignment with zero waste and circular economy policies, however, contain a wide range of chemicals that present an unknown risk of harm in a waste recovery context. Careful assessment of material characteristics, processing methods,

and application to land (including rates, number of applications) is, therefore, needed as part of ensuring minimal environmental harm from chemicals in recovered waste.

Detailed risk assessments have been undertaken for a range of different waste types. Chemical criteria calculations undertaken as part of these risk assessments have used standard methods, where possible, but have also required literature reviews to determine otherwise unknown values for parameters including chemical characteristics and uptake factors into fruits, vegetables, crops and livestock. In addition, criteria to determine if materials are of acceptable quality for resource recovery have been back calculated to determine the concentration of a chemical in a waste material that will not pose an unacceptable risk (as defined in national guidance in Australia) when such waste material is applied to land.

The approaches used to constrain chemical criteria have been applied to a range of waste types and groups of chemicals. Regulators have made use of these detailed risk assessments to set policy and to make regulatory decisions for specific sites. The approaches used to constrain novel chemical criteria will be presented along with the challenges, including variability of chemical concentrations in the wastes and obtaining robust values for critical parameters.

#### **6.04.P-Th403 A Proposal for Ecotoxicological Risk-based Reference Values for Plant Protection Product Residues in Agricultural Soils: Soil Guidance Values**

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The widespread use of Plant Protection Products (PPPs) in agriculture and their persistence in the ecosystem is continuously raising concern about their impact on ecosystem health, including the soil compartment. For PPPs authorization, a prospective risk assessment is performed to guarantee that their application does not lead to unacceptable adverse effects to the soil ecosystem. This assessment is based on single-substance assessment on a limited number of non-target species directly after the application. No retrospective risk assessment is currently performed for evaluating the potential impact of synthetic PPP residues and their mixture in the soil compartment on the long-term.

To fill this gap, we propose a concept where ecotoxicological risk-based reference values will be compared to an annual PPP residue monitoring in in-crop areas. Such values, called Soil Guidance Values (SGV), would serve as generic screening values to identify which sites need further assessment. This further assessment would imply a refinement of the SGV to a site-specific assessment (e.g. considering soil type, climate) and the application of a toolbox of bioindicators to assess the trend of the effects of PPP residues on key soil organisms and functions.

Based on a previous review on several soil regulatory frameworks, specific recommendations are suggested on how to derive SGV with the aim of protecting relevant agro-ecological soil functions in the long term. Some of the recommendations include: the reliability and relevance assessment of ecotoxicological chronic data for in-soil invertebrates, microorganisms and plants considering toxicity only through exposure to PPP residues in soil; the normalization of toxicity data to standard soil organic matter content; and, when possible, the use of ecosystem-relevant derivation methods (e.g. species sensitivity distribution (SSD), mesocosms, field studies) in order to decrease the uncertainty of the effects of the PPP residues in soil ecosystems. In order to show the implications of the recommendations proposed for the derivation of SGV, two PPPs were used as case studies (diuron and fluazinam) and compared to soil reference values derived according to different regulatory frameworks in Europe. Finally, to address the combined toxicity of multiple PPP residues, it is envisioned to develop a concept for a mixture assessment in a further step.

#### **6.05 Environmental Assessment of Nanomaterials, Research to Regulation: From hazard and risk to grouping and similarity approaches**

##### **6.05.T-01 Trimetal-Based Nanomaterials Induced Toxicity to Plants: Does It Differ From Mixed and Single Element Nanoparticles**

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Metal alloys nanomaterials are entering the market and as an example trimetal-based nanomaterials (TNMs) are nowadays used within products. It is essential to raise the question if the toxicity of advanced nanomaterials like TNMs differs from the joint effects as manifested by exposure to the single components. To this end, a trimetal-based nanomaterial: bismuth cobalt zinc oxide (BiCoZnO) was tested and it had a mass ratio of 90% ZnO NPs, 7% Bi<sub>2</sub>O<sub>3</sub> NPs and 3% Co<sub>3</sub>O<sub>4</sub> NPs. Nanoparticle-exposed lettuce seedlings (*Lactuca sativa* L.) showed decrease in relative root elongation (RRE) and biomass after 21 days. The EC<sub>50</sub> values of TNM was 3.0 mg L<sup>-1</sup> and of the same magnitude as the EC<sub>50</sub> values found for ZnO NPs (EC<sub>50</sub> = 2.2 mg L<sup>-1</sup>) and mixture of components NMs (EC<sub>50</sub> = 5.4 mg L<sup>-1</sup>) to lettuce when the endpoint was RRE after 240 h of exposure. The toxicity of ZnO NPs was mostly due to ion release and the actual concentration of released zinc ions was lower for TNMs in comparison to the ion concentrations released by the ZnO NPs. The TNMs tested over time (480 h) gave more toxicity compared to ZnO NPs consisting of one single element. Our results confirmed that the crystal structure of the nanomaterials influenced the dissolution: dissolution was lower than in the single element applications and the toxicity of the metal alloy was also decreased. The metal alloy nanomaterials are having a high synthetic complexity compared to its mixture of single element component NMs, but key principles learned and understood from this study can be applied to aid the effect assessment of trimetal-based nanomaterials.

#### 6.05.T-02 Determination of the Hydrophobicity Index of Nanomaterials: Method, Testing and Applications

**Cloe Desmet<sup>1</sup>, Francesco Roncari<sup>1</sup>, Andrea Valsesia<sup>1</sup>, Marisa Sarria Pereira de Passos<sup>2</sup>, Claudia Cella<sup>1</sup>, Juan Riego Sintes<sup>1</sup> and Pascal Colpo<sup>1</sup>,** (1)European Commission Joint Research Centre, Italy, (2)EU Commission JRC, Italy

Standardised quantitative methods for the determination of hydrophobicity cannot be applied to nanomaterials. A method to fill this gap was developed, which enables the determination of the *hydrophobicity index* (Hy) of NMs. In the context of its submission as an OECD Test Guideline it was tested through an interlaboratory comparison exercise, and its applications as an indicator for environmental behaviour were assessed.

The method is based on a measurement of the affinity of NMs to rationally engineered hydrophobic and hydrophilic surfaces (collectors), monitored by Dark-Field microscopy (DFM). Three types of collectors with different surface properties are produced. Particles are dispersed in an aqueous medium and injected through a fluidic cell. The binding of particles is recorded by DFM and a camera. The number of bound particles as a function of time determines their affinity to the collector. Hy is defined as the logarithm of the ratio between the NMs binding rate on the hydrophobic collector (directly related to the hydrophobicity of the NM), and the one on the hydrophilic collector with a surface charge opposite to the one of the NM (where the binding rate is maximum due to the electrostatic attractive interactions). It is a direct measurement of the tendency of the NM to be attracted to a hydrophobic surface as compared to water. Au, Ag, TiO<sub>2</sub>, SiO<sub>2</sub>, GaN, ZnO and CeO NMs with different types of surface treatment were characterised, as well as nano- and microplastics. The method was tested through an Inter-laboratory Comparison exercise by nine participants, for four materials. The large majority of the results are falling in the  $\pm 1$  STD difference from the average.

The correlation with environmentally relevant parameters was studied for a set of characterised NMs. A batch test experiment was adapted to measure the hetero-aggregation in reference soil. Bioassays were also performed with two freshwater green microalgae presenting different cell wall compositions. Binding, cell growth and oxidative stress were assessed after 24 hours of exposure with the different NMs. The results demonstrated an impact of Hy on the different markers. Further studies should be performed but Hy could provide information on the affinity of NMs to environmental matrices and organisms and help in the environmental hazard and exposure assessment as requested by REACH.

#### 6.05.T-03 Findable, Accessible, Interoperable and Repeatable use of Historical Ecotoxicological Data for Nanomaterial Governance

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Development of novel manufactured nanomaterials is outpacing research capabilities to define environmental risk. Implementing predictive modelling using physicochemical properties is one of the proposed methods to streamline risk assessments for manufactured nanomaterials. The efficacy of this approach hinges on access to robust historical data, which is currently lacking for most ecotoxicological platforms. Centralization of data following FAIR principles can facilitate environmental risk assessment. The aim of NanoInformaTIX is to leverage existing data for ecotoxicity data used in the FP7 project NanoRETox and the Horizon2020 projects NanoREG and NanoREG2 to establish parametric models predicting adverse outcomes. Data were compiled to develop a FAIR database and investigate whether existing data can characterize how physicochemical properties influence bioaccumulation and ecotoxicity for manufactured nanomaterials. Results demonstrated an existing need for standardization in reporting for ecotoxicity data. This presentation highlights the integration of FAIR data practices that can be used to augment predictive modelling in ecotoxicology. This presentation highlights current capabilities of nanomaterial ecotoxicity to address 21<sup>st</sup> century issues and proposes suggestions for FAIRification of ecotoxicological data. This presentation also provides perspectives on database management to maximize scientific investment for contaminants of emerging concern.

#### 6.05.T-04 Towards Harmonisation of Testing of Nanomaterials for EU Regulatory Requirements on Chemical Safety - A Proposal for Further Actions

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Over the recent years, EU chemicals legislation, guidance and test guidelines have been developed or adapted for nanomaterials to facilitate safe use of nanomaterials in society. However, so far the priority needs for development and adaptation of Test Guidelines (TGs) and Guidance Documents (GDs) are largely tailored toward the needs for the adaptation of the REACH legislation. This raises the question of whether other (EU) regulatory areas have specific needs for nanomaterials that have been overlooked in earlier assessments and that may require additional adaptations or developments of OECD TGs or GDs. In this presentation we provide an overview of the information requirements across different EU regulatory areas. For each information requirement, we identified the potential needs (from an EU perspective) for further action to develop or adapt guidance and test guidelines to nanomaterials. In total we identified 136 information requirements across different EU regulatory areas. A need for further action was identified for in total 62 information requirements. Details of these needs are provided in the presentation. Further, the analysis shows that there are multiple overarching issues that are relevant for multiple information requirements and across multiple regulatory areas. These include the needs to 1) resolve issues around nanomaterial dispersion stability and dosing in toxicity testing for especially human health endpoints, 2) further develop tests or guidance on degradation and transformation

of organic nanomaterials or nanomaterials with organic components, and 3) further develop tests and guidance to measure (a)cellular reactivity of nanomaterials. We propose to prioritize work toward three topics. Efforts toward addressing these overarching issues will result in better fit-for-purpose test methods for (EU) regulatory compliance. Moreover, it secures validity of the hazard and risk assessments of nanomaterials. The results of this study underscore the need for increased (innovation) policy – science – regulatory interaction to optimally exploit the full potential of nanomaterials and their contribution to technological solutions to address societal challenges.

#### **6.05.T-05 How to Categorize and Assess “Advanced Materials”: The InnoMat.Life / HARMLESS approach**

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In July 2022 the OECD WPMN released their description of Advanced Materials (AdMa). AdMa are made by rational design, using precise control of its composition and internal structure, and/or are transformed through advanced manufacturing techniques. Advanced Materials have exceptional properties (e.g., mechanical, electric, optic, magnetic) or specific functionalities that differentiate them from the rest of materials (e.g. self-repairing, shape change, transformation of energy). While numerous lessons can be learned from the nanosafety area, it is unclear if nanomaterials are an appropriate point of departure to develop approaches to AdMa risk screening. As another point of departure, the roadmap of the Advanced Materials Initiative (AMI2030) describes the many intended uses and very diverse types of AdMa that industry will commercialize in the coming decade. In the absence of a unifying technological AdMa feature (such as, for nanomaterials, a length scale), a categorization and screening is needed, which must also differentiate AdMa from conventional materials. Here we present such a categorization framework which helps to identify specific concerns for screening and assessment and supports criteria to guide SSbD development of AdMa. The scheme first categorises materials in three dimensions: 1. Does the material consist of particles? 2. Is the material nano-enabled? If consisting of particles, does the revised EU recommendation of a regulatory definition of nanomaterials (2022) identify it as nanomaterial? 3. Manufacturing processes or materials considered as “advanced” identified by several criteria? In this way, the three-dimensional categorisation can be easily displayed as table with four quadrants, and some black (conventional), some red (AdMa) entries. The scheme then asks if the AdMa changes (alleviates or aggravates) the typical SSbD challenges and benefits of a conventional materials that serves the same purpose, as guided by the material categories and industry sectors. To prevent regrettable substitutions, potential new concerns are first addressed by comparing the targeted composition against JRC’s CLP-derived criteria for materials of (very high) concern.

The scheme was compared against other approaches in an OECD WPMN - HARMLESS workshop in November 2022.

#### **6.05.P Environmental Assessment of Nanomaterials, Research to Regulation: From Hazard and Risk to Grouping and Similarity Approaches**

##### **6.05.P-We404 Prioritising Nano- and Microparticles - Identification of Physicochemical Properties Relevant for Toxicity to *Raphidocelis subcapitata***

**Karsten Schlich, Ruben Schlinkert and Kerstin Hund-Rinke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology, Germany**

Advanced/innovative materials are an undefined group of nano- and micro-particles encompassing diverse material compositions, structures and combinations. Due to their unique properties that enable specific functions during applications, there are concerns about unexpected hazards to the environment. By applying algae growth inhibition tests according to OECD 201 with *Raphidocelis subcapitata*, and extensive material characterisation, we aimed to identify indicators of concern. This allows a better prediction of the hazardous properties of these materials in the future. 45 nano- and microparticles of various organic and inorganic species (single constituents and complex compositions; materials releasing toxic ions and others), morphologies (spheroidal, cubic, flaky, elongated/fibrous) and sizes (10 nm – 38 µm) were systematically investigated.

The chemical identity (toxic ion releasing materials vs. other materials) and agglomeration behaviour, which is affected by size (nm vs. µm) and morphology (fibres vs. others), were obvious drivers of ecotoxicity on algae. Differences in morphology had an impact on agglomeration behaviour. Fibres formed agglomerates of varying sizes with entrapped and attached algae. Small compact (e.g., spheroidal) particles attached to algae. A high coverage resulted in high ecotoxicity, while less toxic materials attached to a much lesser extent. No agglomeration of algae and particles was observed for particles with a µm size independent on their chemical identity (organic/inorganic). Small toxic components of large hybrid materials did not affect ecotoxicity. Considering the identified effects and the material characteristics, charts were developed to indicate the expected toxicity of advanced/innovative materials toward algae. Due to the different toxicity mechanisms, materials releasing toxic ions are separated from the other materials. For the former, a dependency on the producer/production process and applied substances not reflected solely by solubility measurements in test media must be considered. For the latter, morphology, size and agglomeration behaviour are considered. Examples for the various clusters are presented, and remaining open questions are defined. The approach can also support read-across and the justification of sets of nano/micro-forms. On the identified criteria three sets of nano-forms for TiO<sub>2</sub> materials are presented and justified by the EC<sub>50</sub> values.

##### **6.05.P-We405 Assessing the Fate and Behaviour of Particulate Emissions From Graphene-based Corrosion-resistant Coatings in Aquatic Environments**

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Novel anti-corrosive coating formulations containing GO and APTES-functionalised GO developed for the aerospace industry were subjected to environmental fate assessment comprising abrasion testing, particulate emission characterization and particulate behaviour studies in aqueous media. Coatings containing APTES/SolGel and GO corrosion inhibitors were applied to aluminium plates (Al 2024 T-3) and subjected to a tribological sliding test (reciprocating TE88-tribometer), which produced dust from all samples and varying damage to coating surfaces. The particulate material generated was characterised for particle size distribution (PSD), shape and morphology. Small (1-20 µm) and large (>100 µm) particles were present in all samples and each coating exhibited a different PSD. SEM images indicated there were no significant differences between any of the coating types. UV spectrophotometry calibration curves were established for GO in synthetic freshwater and seawater (33, 35 and 37 ppm), with and without dispersant (5%). Environmental fate assessment measured (i) sedimentation, (ii) agglomeration and particle size, and (iii) dispersion stability. All end points were measured at regular time intervals up to 330 mins. Studies with the abrasion dust were performed only in freshwater owing to low quantities being available. The GO dispersion was less stable and sedimented more in seawater compared to freshwater, suggesting GO sediments more rapidly in the marine environment and stays in dispersion for a longer in freshwaters. The mean particle size diameter decreased gradually over time, indicating large particles and aggregates gradually settled out. Increased salinity resulted in increasing sedimentation and decreasing dispersion concentration. The dispersant produced a more stable GO dispersion in all media types. In general, mean particle size diameter increased rapidly within a few minutes in freshwater and seawater, before decreasing over time as formed agglomerates sedimented. Zeta potential varied over time, possibly reflecting the continuous removal of larger aggregates through sedimentation. Values remained more stable for freshwater and the dispersant increased GO dispersion stability. Dust abrasion samples exhibited differences in PSD and behaviour over time. GO and APS-GO results in the generation of smaller dust particles which are more stable in aqueous dispersion, while absence of GO produced in larger particles that sedimented quickly.

#### **6.05.P-We406 Embriotoxicity of a Novel Bio-based Nanomaterial with Anti-corrosion Properties on Tropical Marine Species**

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Metallic corrosion is the main cause of degradation of materials immersed in seawater. Currently, this problem is minimized by the addition of corrosion inhibitors (CI) in order to delay the corrosion process. However, such molecules are toxic to the biota, requiring their replacement by eco-friendly alternatives with good anti-corrosion efficacy, such as the biological based corrosion inhibitors (bio-CI), namely sodium gluconate (SG). In the framework of the NANOGREEN project (<https://t.ly/XXi6>), Zn-Al layered double hydroxides (LDH) have been used to immobilize SG, aiming to develop a new generation of anti-corrosion nanoadditives that can simultaneously reduce the typical early leaching of CIs and extend the corrosion protection of metallic immersed substrates over time. Since LDH-SG is a novel nanomaterial, the present study aims at assessing the toxicity of LDH-SG and its free counterparts (SG; Zn-Al LDH) on the embryonic development of two tropical marine species, the sand dollar *Mellita quinquiesperforata* and the mussel *Perna perna*. Organisms were exposed using the standard guideline NBR 15350 with adaptations for *M. quinquiesperforata* (36 h exposure), and NBR 16456 for *P. perna* (48 h exposure) to determine the embryonic development inhibition. For *M. quinquiesperforata* the tested exposure concentrations were 0.41, 1.23, 3.7, 11.1 and 33.3 mg/L, while the *P. perna* the concentrations tested were 1.23, 3.7, 11.1, 33.3 and 100 mg/L. For the three tested compounds, NOEC values ranged between 1.23 mg/L (*M. quinquiesperforata*) and <1.23 mg/L (*P. perna*), while the LOECs ranged between 3.7 mg/L (*M. quinquiesperforata*) and 1.23 mg/L (*P. perna*). For the sand dollar embryos, EC<sub>50</sub> of SG, ZnAl-LDH, and LDH-SG were 37.12 (21.51 - 64.08), 5.766 (3.754 - 8.856) and 1.376 (1.078 - 1.755) mg/L, respectively. In the case of the mussels, EC<sub>50</sub> were 3.739 (2.267 - 6.168) and 0.2645 (0.2198 - 0.3183) mg/L for SG and ZnAl-LDH, respectively; embryos exposed to LDH-SG did not develop in all concentrations tested. These findings contradict recent results obtained for temperate species, for which an opposite trend - soluble CIs are more toxic than the nanostructured forms - has been observed for several invertebrate species. Further studies are advised to confirm the current results and provide sound information to support the development of truly environmentally friendly CIs suitable for all major marine compartments.

#### **6.05.P-We407 Sustainable Nano Strategies for Water Remediation – SENSE Project**

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Sea-level rise and eutrophication threaten freshwater bodies worldwide, posing severe economic, environmental, and human health risks. Coastal areas are in particular danger due to their location and higher urbanization. It is predicted that coastal regions will suffer from seawater intrusion, causing freshwater and terrestrial systems salinization. The excessive nutrient loadings from industrial and domestic effluents and leaching from agricultural fields may increase the risk for eutrophication in these areas, which can consequently cause the deterioration of water quality. Research has been focusing on remediating waters, however, no definitive process has been yet implemented, with some strategies causing deleterious effects to organisms. Therefore, efforts are being made to find environmentally safe alternatives such as nanotechnology-based tools like layered double hydroxides (LDHs). LDHs are anionic nanoclays with ion-exchange properties, composed of positively-charged layers with mixed metal hydroxides, and stabilized by anions and water molecules in the interlayers. The stabilizer anion can be exchanged by anions present in the



surrounding media, such as chlorides or phosphates, making these nanomaterials promising remediation solutions for waters at risk of salinization or eutrophication. The project SENSE aims to provide scientifically based evidence of the efficacy of LDHs to remediate waters potentially impacted by salinization and eutrophication. Waters of the Estarreja region (Aveiro district, northern Portugal) will be used as a case study due to its low-lying coastal lagoon location and historically contaminated waters from the intense industrial and agricultural activities. The experimental design consists of laboratory and *in situ* assays to test the ability of LDHs to remediate chlorides and phosphates from waters, and ecotoxicological bioassays to evaluate the potential toxicity of the remediated waters on freshwater species. Therefore, the outputs of SENSE may provide evidence of the efficient application of LDHs in real scenarios and support its environmentally safe application. These data will be fundamental to help the implementation of this potentially innovative, low-cost, and environmentally safe alternative for water remediation in the market and for improving nanoregulation and risk assessment of these emerging nanoforms.

#### 6.05.P-We408 Are “Smart” Engineered Nanoclays Toxic to Zebrafish?

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Layered Double Hydroxides (LDH) are known as “smart” (i.e., stimuli-responsive) anionic nanoclays or hydrotalcite-like systems and have received a lot of attention in several fields in the last decade due their structures and unique physical and chemical properties. LDH are composed by positively charged mixed-metal hydroxide layers intercalated with anions and water molecules. Also, LDH have versatile physicochemical advantages including excellent biocompatibility, pH-sensitive, high stability and layered structure. The extraordinary properties of LDHs have been exploited in multiple areas, such as nanomedicine (e.g., drug delivery), environmental remediation (e.g., absorbents), industry (e.g., anticorrosive and anti-fouling coatings), among others. Although these nanomaterials are low toxic to humans, mammals and marine organisms, studies focused on the possible effects on freshwater organisms are yet scarce. The zebrafish *Danio rerio* are at the forefront of eco-toxicology research, since it is a well-established freshwater model organism widely used to assess the effects of several chemicals (e.g., nanomaterials, pharmaceuticals). Thus, the present study aimed to assess the physiological, behavioral, and biochemical alterations in *Danio rerio* after exposure to Mg-Al LDH-nitrates. *Danio rerio* eggs were exposed to concentrations ranging from 13 to 100 mg/L of Mg-Al LDH according to the OECD 236 FET protocol, where mortality and malformations were documented daily during 96 h. Following the same principle, neurotoxicity and oxidative-stress related biomarkers, as well as DNA damage (comet assay) were assessed at the end of 96 h exposure. For the behavioral assay, embryos were exposed to Mg-Al LDH for 120 h and locomotory activity of larvae was tracked using the Zebbox (Viewpoint, Lyon, France).

No mortality or malformations were observed at the concentrations tested for the 96 h of exposure. These results provide input for the near-future environmental risk assessment of this nanoclay, showing Mg-Al LDH as a class of promising eco-friendly nanomaterials.

#### 6.05.P-We409 Aquatic Ecotoxicity Assessment to Support the Environmentally Safe Application of Layered Double Hydroxides (LDHs)

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Layered double hydroxides (LDHs) are nanoclays with lamellar structure, composed of positively-charged layers with di- and trivalent metal cations, and stabilized by anions and water molecules in the interlayers. These nanomaterials possess high anion exchangeability and memory effect, making them highly attractive for a wide range of applications, such as in remediation, medicine, and pharmaceutical. Despite being considered an eco-friendly nano-technique, the ecotoxicological assessment of LDHs is in its infancy; therefore, their potential effects on non-target organisms need to be tested. Furthermore, emphasis should be given to studying their potential ecotoxicity towards aquatic species due to their applications in water remediation and antifouling paints. Additionally, the lack of ecotoxicity studies of LDHs needs to be overcome to facilitate their implementation in the market and aid their nanoregulation. Considering this, this study aimed to assess the ecotoxicity of ZnAl-NO<sub>3</sub> and MgAl-NO<sub>3</sub> LDHs (slurry form) in several test model freshwater species: the microalgae *Raphidocelis subcapitata* (algal growth inhibition test; OECD, Test No 201), the macrophyte *Lemna minor* (growth inhibition test; OECD, Test No 221), the water flea *Daphnia magna* (acute immobilization test; OECD, Test No 202) and the zebrafish *Danio rerio* (fish embryo toxicity test; OECD, Test No 236). Overall, results showed that LDHs caused very low toxicity to the test species. These results were compared with our previous data on the ecotoxicity of the calcined and non-calcined forms of the same ZnAl-NO<sub>3</sub> and MgAl-NO<sub>3</sub> LDHs (data presented in SETAC Copenhagen). The toxicity differed between the slurry, calcined and non-calcined forms of each LDH, suggesting that the final form in which the LDHs are produced can significantly influence their ecotoxicity. This is essential information to provide to the manufacturer of these nanomaterials and supports the need for collaboration between academia and industry. Furthermore, this study provides data that may help support the environmentally safe application of these LDHs and might be used for risk assessment and nanoregulation purposes of these nanomaterials.

#### 6.05.P-We410 Comparing Pre-guideline Literature Data on Dispersion Stability for CeO<sub>2</sub> Nanoparticles to Current Guideline Requirements – Can We Use Literature Data for Concluding on the Endpoint and Grouping?

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Dispersion stability is an important parameter determining the environmental fate of nanoparticles. This parameter depends on physicochemical characteristics of the nanoparticles and the suspension medium, as well as on the concentration of nanoparticles and other substances or particles in the medium. It is considered a required endpoint for nanoparticles under REACH, with OECD releasing in 2017 the OECD guideline 318 for standardised testing of dispersion stability of nanomaterials in simulated environmental media and publishing further guidance in OECD Series on Testing and Assessment No. 318 in 2020 (updated in 2021). Obviously, a lot of non-guideline data were generated and published in literature before these OECD documents were published. This is also the case for CeO<sub>2</sub>, where during a literature survey, various studies reporting on dispersion stability of CeO<sub>2</sub> nanoparticles were identified and evaluated. These studies provide evidence for rapid removal of uncoated CeO<sub>2</sub> nanoparticles from natural waters through homo- or hetero-aggregation and consequent sedimentation. They further provide insight into the effect of various conditions on dispersion stability of CeO<sub>2</sub> nanoparticles, such as ionic strength and the presence of natural organic matter, suspended solids, or natural colloids. Although none of these studies were guideline studies, a conclusion was drawn on this mandatory REACH endpoint by setting up a weight of evidence approach including the most relevant studies. In this poster, we are discussing 1) how the (pre-guideline) literature data compare to the current requirements set out in the OECD guidance documents, 2) to what extent conclusions on the endpoint can be drawn in the absence of guideline data, 3) what benefits guideline data would have for the assessment of CeO<sub>2</sub> nanoparticles, and 4) what the implications are for grouping, taking into account the role of dispersion stability in the potential effects in the environment resulting from direct interaction of the nanoparticles with various types of organisms.

#### **6.05.P-We411 A Method for the Production of WWTP-Transformed Silver Nanoparticles for Use in Accumulation and Toxicity Studies**

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Most studies investigating the accumulation and toxicity of engineered nanomaterials (ENMs) utilise pristine test materials. However, in the natural environment, ENMs rapidly undergo a range of transformation processes depending on their intrinsic physicochemical properties and the specific conditions they are exposed to in different environmental matrices. A primary transport route for ENMs from consumer products to the environment are wastewater treatment plants (WWTPs), which represent harsh conditions that can drive transformation of ENM properties. Silver nanoparticles (AgNPs) are known to rapidly sulphidise in WWTPs, changing their properties significantly and therefore more relevant for use in accumulation and toxicity studies. However, producing such materials and isolating them for use in exposure studies is a challenge. In the current study we outline a method for producing bulk quantities of fully sulphidised WWTP-transformed AgNPs for use in such studies. Effluent (10 L) was collected from Høvringen WWTP (Trondheim) and passed through a 0.45 µm filter. The sulphide (HS<sup>-</sup>) content of the filtered effluent was then determined iodometrically. A stock solution of HS<sup>-</sup> was prepared using sodium hydrosulphide hydrate (NaHS) and the concentration also verified iodometrically. In the next step, 10 mg of AgNPs were added to 10 L of filtered WWTP effluent and the sulphidisation process allowed to proceed. The remaining free HS<sup>-</sup> concentration was determined iodometrically every 2 hours over a 6 hour period until a stable concentration was recorded or until all the HS<sup>-</sup> had been reacted. Where all the available HS<sup>-</sup> has been reacted, full AgNP sulphidisation is assumed to be incomplete. In this case, the HS<sup>-</sup> stock solution is added to the AgNP-effluent solution at a concentration calculated to be sufficient to sulphidise all the AgNPs present. Further sulphidisation of the AgNPs is allowed to proceed (up to 6 hours) and then the HS<sup>-</sup> concentration determined iodometrically to ensure that a stable excess of HS<sup>-</sup> is present. At this point, the fully sulphidised AgNP-effluent solution is ready for utilisation in accumulation and toxicity tests using aquatic organisms. The approach is scalable to large volumes of effluent water allowing assessment of WWTP-transformed AgNPs in flow through systems for accumulation and toxicity testing.

#### **6.05.P-We412 Environmental Behavior and Hazard of a Novel “Smart” Engineered Nanomaterial**

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Layered double hydroxides (LDH), also known as anionic plate-like nanoclays, are a class of stimuli-responsive (or “smart”) inorganic engineered nanomaterials. They have been used in several industrial and material engineering applications due to their physico-chemical properties, such as high surface area and capacity to control the exchange of a wide range of anions, depending on the presence of specific triggers (e.g. pH changes). The effects of LDHs, and, in particular, Mg-Al LDH stabilized with nitrates (Mg-Al LDH-NO<sub>3</sub>), have been scarcely or even not yet studied in marine organisms. Therefore, this study aims at assessing the behavior in seawater, the exposure effects, and the marine hazard of Mg-Al LDH-NO<sub>3</sub>.

The behavior of Mg-Al LDH-NO<sub>3</sub> was assessed in terms of electrochemical stability (through zeta potential), hydrodynamic size (dynamic light scattering), anions release (nitrates spectrophotometric quantification), and dissolution (Mg and Al determination, ICP-OES). Ecotoxicological tests were carried out using 14 marine species from different trophic levels: bacterium (*Aliivibrio fischeri*, *Idiomarina seosinensis*, *Halobacillus locisalis*), microalgae (*Chaetoceros calcitrans*, *Isochrysis galbana*, *Phaeodactylum tricorutum*, *Tetraselmis chuii*), rotifers (*Brachionus plicatilis*), bivalves (*Scrobicularia plana*, *Mytilus galloprovincialis*), crustaceans (*Artemia salina*) and echinoderms (*Paracentrotus lividus*). Exposure concentrations ranged from 0.41 to 100 mg/L (dilution factor of 3), depending on the species, plus a negative control of artificial saltwater. The lowest no observed effect

concentration (NOEC) was then used to derive the predicted no effect concentration (PNEC) dividing the NOEC by an appropriate assessment factor (100).

Aqueous dispersions of Mg-Al LDH-NO<sub>3</sub> are unstable. In artificial saltwater, larger aggregates tend to sink in the first 24 h, particularly in the highest tested concentration (100 mg/L). The release of nitrates is fast in the first hours of exposure until reaching a plateau. Dissolution of Mg-Al LDH is minimal over the exposure period. This nanomaterial was not toxic to all tested species (lowest NOEC=0.41 mg/L for *P. lividus*). The PNEC for Mg-Al LDH-NO<sub>3</sub> was set at 4.1 µg LDH/L. The study suggests that Mg-Al LDH is a safe and sustainable by design nanomaterial. These insights will be critical for academic and regulatory purposes and will contribute for the implementation of the EU Chemicals Strategy for Sustainability.

#### 6.05.P-We413 “Smart” Anti-Corrosion Nanomaterials: What We Know About Them?

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Layered double hydroxides (LDH) are regarded as “smart” or stimuli-responsive engineered nanomaterials due to their anionic exchange capacity. The anion nitrite has an outstanding anti-corrosion performance in steel substrates and its immobilization in Mg-Al or Zn-Al LDHs can be used to mitigate the early leaching of conventional corrosion inhibitors. Despite their industrial relevance, the fate, behavior and hazard of Mg-Al LDH-NO<sub>2</sub> or Zn-Al LDH-NO<sub>2</sub> in the marine compartment are not yet known. Therefore, this study aims at presenting the physical and electrochemical characterization and the behavior and hazard of Zn-Al LDH-NO<sub>2</sub> and Mg-Al LDH-NO<sub>2</sub> in the marine compartment.

Physical and electrochemical characterization included scanning electron microscopy, X-ray diffraction and impedance measurements. Environmental behavior in artificial saltwater (ASW) included colloidal stability (zeta potential), hydrodynamic size (dynamic light scattering), nitrites release (spectrophotometric quantification), and dissolution (Zn, Mg and Al determination, ICP-OES). Ecotoxicological tests were carried out using bacterium (*Aliivibrio fischeri*, *Idiomarina seosinensis*, *Halobacillus locisalis*), microalgae (*Chaetoceros calcitrans*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Tetraselmis chuii*), rotifers (*Brachionus plicatilis*), molluscs (*Mytilus galloprovincialis*, *Phorcus lineatus*, *Scrobicularia plana*, *Steromphala umbilicalis*), crustaceans (*Artemia salina*) and echinoderms (*Paracentrotus lividus*). Exposure concentrations ranged from 0.41 to 100 mg/L (dilution factor of 3), depending on the species, plus a negative control of ASW.

The immobilization of nitrites into LDH was confirmed through XRD and chemical analyses. Aqueous dispersions of both LDH are unstable. In artificial saltwater, larger aggregates tend to sink in less than 24 h. Tested LDHs are not toxic under the range of tested concentrations ( $E/I/LC_{50} > 100$  mg/L) for most species, except for *P. lividus*, *I. galbana*, and *P. lineatus* ( $E/I/LC_{50} = 5.4$ , 45.2, 93.0 mg/L, respectively), in the case of Zn-Al LDH-NO<sub>2</sub>, and *I. galbana*, in the case of Mg-Al LDH-NO<sub>2</sub> ( $IC_{50} = 38.4$  mg/L). The sea-urchin *P. lividus* set the lowest NOEC (<0.41 mg/L) for both LDHs, a value that does not allow the successful derivation of the hazard endpoint. Despite this, the Mg-Al form seems safer, and such findings contribute to increasing the foreground knowledge towards a new generation of efficient and truly eco-friendly anti-corrosion additives.

#### 6.05.P-We414 Direct Quantification of Hydrophobicity: A Case Study of Environmentally Relevant Silver Nanoparticles

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Among the physical and chemical properties of nanomaterials, hydrophobicity is recognized to play a key role in their environmental impact. This parameter is involved in abiotic and biotic processes and could be used in the prediction of nanoparticles' (NPs') behaviour in the environment, in terms of aggregation, toxicity and bioaccumulation. Through a dark field microscopy (DFM)-based method, this paper characterized the change in hydrophobicity of silver (AgNPs) induced by sulfidation and by the formation of a corona of natural organic matter (NOM). DFM results, measured as a Hydrophobicity index (Hy), were compared with the dye adsorption method results (log HR).

The sulfidation of the AgNPs and/or the adsorbed NOM/ “Lipid-free” (LF-NOM) increased their own hydrophobicity, increasing the Hy values. The tendency of sulfidized AgNPs to become more hydrophobic is demonstrated by both methods. Log HR results were not able to differentiate the hydrophobicity of AgNPs with varying natural organic matter (NOM) coronas. This study shows that the DFM-based method, can effectively measure the hydrophobicity of environmentally relevant NPs and it has a potential to be used as fate predictor.

#### 6.05.P-We415 Using a Meta-analysis Approach to Assess Silver Nanomaterial Hazard to Terrestrial Soil Ecosystems for Safety-by-design Practices

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Extensive research has been conducted on the ecotoxicological risks of silver nanomaterials (AgNMs) on freshwater organisms and hazard thresholds are established based on species sensitivity distributions (SSD). Similar hazard thresholds for released AgNMs for terrestrial soil organisms have not been so well established. To address this knowledge gap, this study collected existing dose-response data of different Ag forms from the literature, including a benchmark (AgNM-300K), ionic (AgNO<sub>3</sub>) and environmentally relevant (Ag<sub>2</sub>S) forms of Ag. For AgNM-300K, 546 soil toxicity values were gathered in a database from 29 articles published between 2013-2021, across soil-dwelling organisms. Data availability by taxa followed the order microbes >

enchytraeids > springtails > earthworms > nematodes > plants. Alongside toxicological endpoint data, the database captured exposure characterisation and AgNM fate measurements within the exposure medium. This allowed variation in toxicological endpoint data across species endpoints for the different Ag forms to be assessed, including the influence of environmental parameters on driving Ag toxicity. To obtain estimates of hazardous concentrations affecting 5% of species (HC<sub>5</sub>), SSDs based on predicted no effect concentrations (PNECs) were constructed. Results show that AgNM-300K produced higher HC<sub>5</sub> values when aged (HC<sub>5</sub> = 0.05 mg/kg) compared to 'as manufactured' state (HC<sub>5</sub> = 0.03 mg/kg) and to AgNO<sub>3</sub> (HC<sub>5</sub> = 0.04 mg/kg) in soils. In relation to existing soil toxicity thresholds for persistent, bioaccumulative and toxic substances, AgNM-300K HC<sub>5</sub> concentrations fall within the 'very toxic' category (PNEC < 0.1 mg/kg). In a safe-by-design context, the difference between primary particle characteristics and their hazard need to be elucidated, and this approach demonstrated that although differences may be detected, the chemical composition drives hazard more compared with particle properties for soils. Further to establishing the most up to date HC<sub>5</sub> values for Ag in soils, we were able to use the database to explore apparent reproducibility of commonly used assays tested in the same species and under similar conditions and identify species most sensitive to this nanomaterial. This knowledge can support recommendations for sensitive and reproducible assays suited for inclusion in soil hazard assessment, with regulatory relevance and for guiding a safety-by-design approach for manufactured NMs for the protection of soil ecosystems.

## 6.06.P Evidence Led Approaches to Identifying Chemical Priorities and Groupings for Effective Risk Management

### 6.06.P-We416 A Method for Ranking and Prioritising Chemicals Without The Need For Significant Resources

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We present a methodology designed to rank hazardous chemicals without requiring the user to expend significant computational or human resources.

The ability to compare chemical toxicity is increasingly problematic for the non-expert. Current existing methods require manual collection of data and expert knowledge to interpret. Inconsistent methodologies, gaps in available data, and outdated information seriously hamper attempts to compare like with like. In addition, the volume of chemical information released on a daily basis surpasses the ability of resources to process it.

Data, incorporating select environmental toxicity parameters and human health impacts, are drawn from internationally recognised chemical databases such as the United States Environmental Protection Agency (USEPA) and the European Chemical Agency (ECHA). Algorithms enable users to quickly and simply refine groupings of chemicals to a high priority subset, based on customisable filters. The curated and qualified chemical information enables substance comparisons via transparent and uniform hazard criteria and provides unique visuals to facilitate communication of results to stakeholders.

This method does not replace or supersede existing systems used to make regulatory or other decisions. Rather it enables users to rapidly identify and refine areas for further research, thus maximising resource allocation.

### 6.06.P-We417 Identification, Grouping, and Prioritisation of Water-Soluble Polymers in Household Products

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Polymers are long-chain molecules which typically exist as a distribution of homologues across a range of molecular weights. Due to their large size, polymers have previously been exempt from regulatory initiatives such as REACH. However, given their widespread usage and environmental emissions, there is a need to incorporate polymers into environmental risk assessment schemes. The huge number and variety of water-soluble polymers (WSPs) in current use presents a significant challenge for risk assessment, especially given the lack of transferability of many existing assessment methods. In addition, data on formulation, usage volumes, and even substance identity and composition, remain unavailable for many polymers. It is thus essential to develop approaches for identifying, grouping, and prioritising WSPs in current use. Here we present a prioritisation approach for identifying WSPs in current use that have the greatest exposure and pose the greatest risks to the environment.

An inventory of polymers found in household products was built using publicly available ingredients data. Polymers were grouped by chemical structure and function in products, and formulation concentrations were estimated from patents. Estimates of market penetration were based on frequency of occurrence in the studied products. These data were combined with literature usage data to obtain worst-case predicted environmental concentrations (PEC). PECs for the top ten polymer groups were further refined based on wastewater treatment fate from literature data, and resulting PEC estimates were combined with predicted no-effect concentrations to obtain risk quotients (RQs) for prioritisation.

A total of 339 individual polymers were identified across 1,353 products and subsequently divided into 26 groups. Based on limited ecotoxicity data, estimates of aquatic RQs indicate that polyethers and copolymers, polyquaterniums, and polyol ethoxylate esters (e.g. polysorbates) may be of highest potential concern. This study provides key knowledge of the types of WSPs which may be present in the environment, a method for identifying these and other ingredients and estimating usage volumes from publicly available data without prior knowledge of chemical identity, and a basis for grouping of WSPs for risk assessment, as well as initial estimates of environmental exposure and risk.

### 6.06.P-We418 A National Scale Prioritisation and Early Warning System for Chemicals of Emerging Concern

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The UK Government's 25-year Environment Plan highlights the need for early identification of chemicals of concern, so that effective intervention can be undertaken prior to damage being caused to the environment, wildlife or human health. To address

this chemical challenge the Environment Agency (EA) have developed a Prioritisation and Early Warning System (PEWS) for England. Our aim has been to establish a system which ensures the appropriate regulatory focus on individual and groups of substances of emerging concern. This is based on a suitable level of risk assessment, considering each substance in terms of readily available use, fate and ecotoxicological/toxicological parameters and monitoring data.

The PEWS process includes nomination and sifting of substances, screening of substances for potential risks to human health via the environment, and to surface waters (both freshwater and marine), groundwater, soils, biota and sediments. Chemicals are then prioritised for environmental regulation if there is considered a sufficient level of risk. For some substances, the process demonstrates a need for further human biomonitoring and environmental monitoring to help understand current concentrations and increase certainty in prioritisation.

In this presentation we describe the progress of the development and use of a National scale early warning system. We illustrate how use of the system furthers our understanding of substances which have been screened for potential risks to date, and the link from PEWS to potential regulatory intervention, where this process has shown that to be appropriate for a chemical. We demonstrate a case study of veterinary medicines in the environment, showing the connection from assessing the science to identifying regulatory gaps. We highlight the outstanding technical challenges and some of the approaches that we are undertaking over the year ahead to overcome these.

#### 6.06.P-We419 Harmonizing Non-Target-Screening Results for Aggregated Analysis

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Non-Target-Screening (NTS) by high-resolution mass spectrometry in surface water is widely used for the monitoring of organic pollutants. At the moment, datasets often remain locally isolated and pooling data to help the identification of unknown detections is slow and not scalable. The current project sets out to develop a system of harmonizing and linking *pre-processed* NTS data from different methods and software through the use of 1) a standardized format for the *processed* results, 2) harmonization scripts to convert existing data to this format and 3) an algorithm to compare all data and link detections of the same compound, so-called alignment. To make use of this harmonized data, a frontend web-application (*NTSPortal*) was developed to allow searching of the data and displaying maps (spatial distributions) and time-series of the aggregated results. A common web-standard data serialization format (JSON) was chosen for the standard format for the processed NTS data. This format is the standard format for many database systems and search libraries, allowing existing software to be used. For this project, ElasticSearch was used to make the data searchable. A harmonization script to transform processed data into JSON was written in R. The alignment process was performed on the data after it was loaded into ElasticSearch. An R package was written to recall individual features (detections) from the database, make comparisons by clustering using *m/z*, retention time and MS<sup>2</sup> spectral similarity, and write back to link features by using common IDs. To validate the whole procedure, several metrics were defined to help assess the quality of the alignment. The two most important ones being: a) Fraction of “orphan” features and b) Fraction of “falsely grouped” features. The database currently contains 1.3 million features from 1087 measurements, 12 sampling stations across Germany and 2 matrices (water and suspended particulate matter). These were clustered into 17,854 grouped features. 70% of features had an associated MS<sup>2</sup> spectrum. Of these, 26% were “orphans” and 7% were “falsely grouped”. Using the interactive web front-end, a user can search for a feature by attributes such as *m/z* and MS<sup>2</sup> fragments and see the occurrence patterns of matching features regionally and at a selected sampling stations over time. These first experiments show the feasibility of aggregating processed NTS data from across different regions and matrices.

#### 6.06.P-We420 A Combined Passive Sampling and Non-Targeted Analysis Approach to Prioritise Organic Pollutants in Coastal Environments

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Coastal environments will be exposed to hundreds of chemicals arising from multiple sources, but only a proportion of these are likely to cause ecological harm. Therefore, there is a need for an approach specific to marine ecosystems, so that future work can be targeted at those substances that pose a potential hazard and risk. Here, we present a prioritisation framework combining passive sampling using Chemcatchers<sup>®</sup>, non-targeted LC-QToF MS analysis and QSAR modelling to prioritise organic chemicals in the Firth of Forth (Scotland) in terms of their potential risk to the European shag (*Gulosus aristotelis*). Chemcatcher data were collected from two sites in proximity to European shag colonies during 2021. Seven datasets relevant to the exposure pathway were chosen to inform three parameters: persistence (P) (qualitative DT<sub>50</sub> data for sediment and water); bioaccumulation (B) (bioconcentration factor for higher trophic levels); and acute toxicity (T) (quantitative fish LC<sub>50</sub> values and an extended Cramer classification for acute human toxicity). Using these data, each chemical was attributed to one of seven prioritisation categories (vB-vP-T; P-vB-vT; vP-B-vT; vP-vB; vB-vT; vP-vT; P-B-T) based on EPA and REACH guidelines. For comparison, the same method was repeated for 111 POPs that are to be monitored in the same study system. In total 404 chemicals were detected, with data obtained for 395. Of these, 17/395 chemicals fell into either vB-vP-T, P-vB-vT or vP-B-vT categories, with 13 specifically vB-vP-T. A further 113/395 chemcatcher chemicals were categorised as either vP-vT or vT-vB, whereas no chemicals were assigned to either the vP-vB or P-B-T category. Comparatively, 50/111 POPs were assigned to the vB-vP-T category and 37/111 to either vP-vT or vT-vB. The majority of vB-vP-T chemcatcher chemicals contained either a long hydrocarbon chain or at least one benzene ring, whilst the structure of non-prioritised chemicals was varied. This framework identified 17 chemicals that had similar P-B-T properties to well-known classes of POPs, such as PCBs and PBDEs. In particular, 13 chemicals were classed as

vB-vP-T and will be taken forward into a spatial bioaccumulation model to assess their potential impact on European shags resident at both sampling sites. With many QSAR models available, this approach is malleable to a range of mixed-chemical exposure pathways to prioritise and identify potential contaminants of emerging concern in coastal environments.

#### **6.06.P-We421 Ad hoc Assessment of Non-target Screening Data in the Aquatic Environment: Prospects and Challenges for Science and Regulation**

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Analytical advances using high-resolution mass spectrometers such as suspect- and non-target screening (NTS) provide valuable information on contaminants of emerging concern (CECs) in the aquatic environment. NTS data are challenging for authorities as regulatory guidance is still missing for the use of semi-quantitative data. Monitoring programmes, in turn, frequently lack regulatory information on CECs such as regulatory status, sale/production volumes or hazard information. The myriad of chemicals on the European market currently outpaces the capacity of chemical regulators to assess hazards and risks for many CECs. A recognised problem is that testing requirements for the placing of chemicals on the market in Europe are not harmonised between chemical legislation. Up to now, there is no centralised database available that provides regulatory information on chemicals irrespective of their legislation. In Germany, the German Environment Agency (UBA) represents the competent authority for environmental risk assessment in chemical legislations. In the project *water monitoring of the future*, we aim to combine NTS data from the aquatic environment with regulatory information from UBA in an Elasticsearch-based database called NTS Portal. The ultimate goal is to provide an ad-hoc risk assessment of CECs independent of their respective uses by linking monitoring data (e.g. detection rates), use information (e.g. tonnages) and hazard-based parameters from OECD/ISO guidelines (e.g. PBT/PMT, fate, effects). In addition to the assessment of individual substances we also aim to characterise priority mixtures, point sources and trends in water and suspended particulate matter samples. Here we provide examples for an ad hoc assessment of substances that were identified and prioritised using established NTS workflows and compare our approach to established prioritisation approaches, e.g. from NORMAN. Linking regulatory information with monitoring data in a centralised database is crucial for dealing with the myriad of registered/authorised substances on the European market and will ultimately strengthen the connection between regulation and science.

#### **6.06.P-We422 Substantiating Chemical Groups for Read-Across Using Molecular Response Profiles**

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The chemical grouping and read-across paradigm is a key approach to support the evaluation of environmental and human health hazards posed by chemicals, whilst reducing or even avoiding animal testing. It provides a process by which toxicity endpoint data can be read across from a data-rich to a data-poor substance(s) and is underpinned by the concept that structurally similar substances generally elicit similar toxicological effects. However, structural similarity alone is typically not sufficient, and additional supporting data such as a shared mode of action can strengthen a grouping hypothesis and hence justify a read-across. The overall aim of this study is to demonstrate how multi-omics measurements can be used to increase confidence in a grouping hypothesis by using bioactivity signatures that are reflective of a chemical's mode of action.

We investigated six substances with two known modes of action, including three phthalates; benzyl butyl phthalate (BBP), dibutyl phthalate (DBP) and diisobutyl phthalate (DiBP) and three uncouplers of oxidative phosphorylation; 2,3,4,5-tetrachlorophenol (TCP), carbonyl cyanide 3-chlorophenylhydrazone (CCCP) and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP). Conventional structure-based grouping approaches were first applied to formulate a grouping hypothesis. Next, short-term exposures were conducted in *Daphnia magna* at EC<sub>10</sub> (48 h immobilisation) concentrations to produce multi-omics data (LC-MS metabolomics and TempO-Seq transcriptomics). A bioactivity-based grouping—measuring the similarity between the 'omics responses—was then derived from these data using hierarchical cluster analysis.

Conventional structure-based grouping approaches struggled to assign the uncouplers and phthalates into two anticipated groups with the structurally diverse uncouplers often assigned into 2–3 different classes. Despite varying magnitude in the number of perturbed features between treatments, bioactivity-based grouping using multi-omics data placed the substances in two distinct groups, one containing the uncouplers of oxidative phosphorylation and the other the three phthalates.

This study demonstrates that molecular (multi-omics) data can successfully be applied to calculate substance similarities, thereby increasing confidence in category formation with biologically-based knowledge. This finding is relevant to chemical risk assessment to increase the efficient use of available data.

#### **6.06.P-We423 Demonstrating the Reliability of Metabolomics-based Chemical Grouping: Towards Acceptable Practice**

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Metabolomics has reached a critical point in determining its value to regulatory toxicology. Building on 20 years of research, the

first metabolomics data to support grouping/read-across was recently submitted to ECHA, metabolomics best-practices for various applications including grouping were published in *Nature Communications*, and the OECD Omics Reporting Framework has been developed. Given these and other examples of its increasing relevance to chemical safety regulations, an assessment of the reproducibility of metabolomics in the context of chemical grouping is required. The aim of the Cefic-funded MATCHING study (MetAbolomics ring-Trial for CHEmical groupING) is to determine whether this technology can demonstrate high reproducibility in grouping, and hence high reliability, or whether refinements in analytical or data analysis practices are needed. Through this fully-blinded evaluation, the second aim is to propose ‘acceptable practice’ for metabolomics-based grouping. The international consortium comprises seven industrial, government and academic metabolomics ring-trial partners, BASF SE, and the European Chemicals Agency (ECHA) as an independent advisor. First, 8 substances were selected for the trial, and all ring-trial partners were fully-blinded to their identities, modes of action, and the number of categories. Plasma samples for the ring-trial were then derived from 28-day rodent tests (8 substances, each ‘low’ and ‘high’ dose, plus vehicle controls), aliquoted, and distributed to partners. Each metabolomics laboratory then applied their preferred LC-MS metabolomics workflows to acquire, process and statistically analyse the plasma samples. This included attempting to group the 8 substances into categories based on their metabolomics signatures, and then reporting their findings to ECHA to ensure the blind conditions of the trial were respected. To date, five ring-trial partners have reported, and all have discovered the identical grouping of the 8 substances into 3 unique categories (remaining two reports expected by December 2022). Further analyses into the metabolic biomarkers driving the groupings are underway. In addition, both the consistent and differing elements of the consortia’s metabolomics workflows are being examined to propose acceptable practice for metabolomics-based grouping. In conclusion, the findings from the MATCHING study have demonstrated high reliability of metabolomics-based chemical grouping.

## 6.06.V Evidence Led Approaches to Identifying Chemical Priorities and Groupings for Effective Risk Management

### 6.06.V-01 Converging Degradation Pathways as a Basis for Grouping & Read-Across

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Since the REACH legislation, Read-Across became a frequently used option for predicting toxicity and properties of chemicals. While (Quantitative)-Structure-Property-Relationships ((Q)SPR) have achieved some regulatory acceptance (e.g., for concluding of explosive properties or estimating vapour pressure of substances with a melting point above 200 °C), the derivation of biological effects is often not very solid and gets refused.

Formaldehyde Releasers are a good example for a straight-forward applicable Read-Across. The cleaving of formaldehyde is often intended and an important primary degradation step, while the remainder of the parent molecule normally does not degrade very fast and shows little bioactivity. Nonetheless, more complex grouping may be based on converging pathways where the relevant metabolite or transformation product is formed after some antecedent degradation steps may be an option. As an illustration of the principle, the poster shows an example: A variety of substances are potentially able to converge in the metabolic pathway of benzene, i.e., the formation of Hydroxyhydroquinone, potentially the critical molecule in the benzene toxification (metabolic activation).

## 6.07 Getting the Soil Loop Rolling: Ecotoxicology, Risk Prediction, Monitoring and Back

### 6.07.T-01 ERAMYC - Assessing the Sensitivity of *Arbuscular mycorrhizal* Fungi to Chemicals in Soil

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Arbuscular Mycorrhizal Fungi (AMF) are organisms that live in symbiosis with most plant species, being responsible for supporting key soil functions and providing important ecosystem services. Due to their ecological relevance and sensitivity to chemicals, AMF have been indicated by the European Food Safety Authority (EFSA) as a potential group of non-target test organisms to be used in the risk assessment of Plant Protection Products.

The existing ISO protocol 10832 describes a method to evaluate the effect of chemicals on AMF, but considers only spore germination (pre-symbiotic phase). Germ tube formation and asymbiotic hyphal growth are important phases of the fungal life cycle and are essential for establishing symbiosis. However, other processes like root colonization could be affected by chemicals in the soil and are not investigated when following the existing test protocol.

ERAMYC is a project which integrates a consortium of experts with the aim to 1) improve the existing protocol for the pre-symbiotic phase by including additional AMF species, test conditions and procedures embracing the symbiotic phase of AMF species; 2) standardize the developed test protocol through a ring-test with different chemicals; 3) develop a draft OECD Test Guideline and evaluate experimental results in reference to the existing framework for the risk assessment of soil organisms exposed to chemicals.

To comply with these objectives, two phases of preliminary experiments were performed. In the first phase, experiments were conducted without chemicals to evaluate the performance/suitability of the selected AMF species in different soils under specific environmental conditions and selected host plants. The second phase included the use of test chemicals to assess how the different AMF species and respective parameters react to the selected chemicals.

The use of seeds pre-soaked in water and a total AMF colonization higher than 40% in controls have been required to validate the experiments. Results of previous studies have shown that Chlorothalonil had effects on both the pre-symbiotic and symbiotic

phases in the AMF *Gigaspora albida* and *Rhizophagus clarus*. In the symbiotic phase, arbuscular colonization was, in most cases, more sensitive when compared to total colonization and total extra-radicular mycelium length. However, this pattern can be different when testing an herbicide or/and a fungicide with a different mode of action.

#### **6.07.T-02 The Influence of Soil Organic Matter Content on the Toxicity of Pesticides to Soil Invertebrates: A Review**

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Lipophilic organic chemicals can adsorb to soil organic matter (SOM), reducing their bioavailability to soil invertebrates. As such, the European Food Safety Authority (EFSA) suggests that for lipophilic pesticides ( $\text{Log Kow} > 2$ ), the endpoints derived from laboratory toxicity tests performed in artificial soil containing ~10% SOM should be divided by a factor of 2 to correct for the lower SOM contents in agricultural soils (~5% SOM). These corrected endpoints can then be used in the subsequent risk assessment. Recently, the application of this factor of 2 has been questioned, as it is based on 14-day earthworm toxicity tests focussing on mortality, instead of 56-day earthworm toxicity tests focussing on reproduction. As such, its suitability for other standard soil test invertebrates (Collembola, Enchytraeidae, Acari), and toxicity endpoints (i.e. reproduction) is questionable. A better understanding of how SOM content influences pesticide toxicity to soil invertebrates is needed to improve the ecological relevance of risk assessment approaches. In the current study, soil invertebrate toxicity data ( $\text{LC}_{50}$  &  $\text{EC}_{50}$ ) were collected from studies determining the toxicity of organic chemicals in soils with varying SOM content. Relevant studies were identified by performing a literature search and through the use of toxicity databases. The data were used to address the following questions: 1) Can the relationship between toxicity and SOM content be quantified?, 2) Does SOM content influence different toxicity endpoints in a similar way?, and 3) Is the influence of SOM content on sensitivity to pesticides different between species? The results indicate that toxicity-SOM relationships are chemical dependent, not clearly different between lipophilic and non-lipophilic chemicals, differ between endpoints, and are species specific. Hence, the grouping of chemicals based solely on their lipophilicity, as well as having only one correction factor for multiple species, may not be an appropriate approach in risk assessment.

#### **6.07.T-03 How Could the New Soil Exposure Framework Be Implemented into a Future, Tiered Ecotoxicological Testing Scheme?**

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In the EU risk assessment (RA) for soil organisms exposed to plant protection products (PPPs), laboratory derived ecotoxicity endpoints are compared with the predicted environmental concentrations in soil at lower tier. If a potential risk is indicated, higher tier field studies are conducted to investigate whether effects on soil organism communities occur at relevant field application rates under natural conditions.

As EFSA published a new guidance for the exposure assessment of PPPs in soil in 2017, an impact assessment has been conducted by CropLife Europe to investigate possible changes in exposure values of active substances and potential consequences for the soil RA. Key outcomes were that considerably higher exposure values can be expected in all regulatory zones compared with the current FOCUS modelling. Increase was correlated with specific soil parameters (including bulk density and organic matter (OM) content), pre-defined wash-off fractions and obligatory model and scenario specific correction factors.

Consequently, a significant increase in the number of active substances failing the lower tier soil RA was observed, triggering higher tier field studies as a refinement.

High soil exposure values were often related to high OM levels in the exposure scenarios with median values of 6.1, 10.3 and 19.4 % in the southern (SEU), central (CEU) and northern (NEU) zone, respectively. However, these median values assumed in EFSA modelling are clearly higher compared to other, measured OM data for EU, and compared to higher tier field studies conducted on typical agricultural soils in CEU, with a median OM content of 3.1 % ( $n = 54$ ). Further, toxicity endpoints from the laboratory are corrected for lipophilic compounds ( $\text{Log}_{\text{Pow}} > 2$ ), assuming that toxicity is underestimated in soil with high peat content (and high OM). Thus, direct comparison of exposure and effects may thus under- or overestimate the risk to soil organisms.

To properly refine soil RA based on exposure values from the new framework, a joint understanding and consideration of both, exposure parameters and ecotoxicological effect characterisation is required. Adjustment of modelling soil characteristics to real agricultural soil characteristics, e.g. by using natural soils in an intermediate tier testing, could, together with a reduced assessment factor, quantify the potential risk more realistically and serve as an additional step before the initiation of field studies.

#### **6.07.T-04 Soil Health As Part of Sustainable Agricultural Production Systems**

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Soil health is considered an integral part of sustainable agriculture, therefore requiring special attention by farmers when setting up their farming practices. Within the agricultural production context, soil health interacts with the various other tools and methods that are used by farmers to create a successful cropping situation and sustainable production system.

Along other sustainability criteria, soil health properties with focus on nutrient balance, organic carbon and earthworm populations have been investigated on two farms in Germany, taking into account the challenges and opportunities of different farming systems and approaches with the aim to improve their sustainability profile, respectively, over the five years of the planned trial period.



While the sustainability is assessed across several key indicators, such as crop yields per ha to reflect the land use footprint, the crop quality, economic assessment of the production scenario, greenhouse gas emissions per volume crop produced, biodiversity of carabids as proxy for natural pest control and biodiversity and their diversity and biodiversity of plants in habitats adjacent to the fields, soil health is specifically considered with focus on nutrient balance, organic carbon and earthworm populations. The soil health implications within the current crop rotations are presented. The yields, climate and further environmental implications of the different production scenarios with their corresponding choice of tools and methods have been simultaneously assessed, thus enabling a holistic perspective that takes into account the farmers agronomic challenges, decision-making and opportunities for improvement.

The further assessments are taking place over an extended crop rotation, following the corresponding agronomic requirements and the practical implications for the farmer with the aim to cover five subsequent years and to continuously improve the sustainability of the scenarios over time. Along with the recent performance challenges are presented that could appear currently as limiting factors for an improved monitoring or hint towards trade-offs.

#### **6.07.T-05 Assessing the Effectiveness of Risk Mitigation Measures on the Risk Values of PPP Spray Series: A Case Study with Soil Organisms on Vineyards**

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To reduce the impact of Plant Protection Products (PPPs), risk mitigation measures (RMM) are usually suggested and implemented. Some RMM are related to the use of more efficient and target-specific pesticides, the use of new spraying technologies, the development of precise Early Detection Systems (EDS) and of Decision Support Systems (DSS) that reduces the PPP's use by predicting disease outbreaks. These RMM were developed/tested in isolation and in combination in field trials at the H2020 OPTIMA project aiming to develop an optimized novel Integrated Pest Management (IPM) system targeting the goals of the Farm to Fork strategy. In this study, we assess the risk of PPP spray series used in vineyards to earthworms, where those RMM measures were adopted. The aim was to holistically assess the cumulative risk of each spray series and the effectiveness of the RMM (isolated or in combination) in reducing the risk to in-soil organisms. Six field trials (named Ref 0 to Ref 5) were conducted on vineyards (Barbera variety) in Italy, where the different RMM were tested. Each reference consisted of a spray series with 13 application events, where 19-20 active substances (a.s.) were used. Ref 0 was considered the baseline condition. The risk of the spray series from each reference was calculated deriving the daily cumulative ETR values (ETRsum) over the application period of 144 days based on the CA model. The toxicity data (NOEC) were collected in DAR/RAR or derived from laboratory tests. PEC values were estimated using the PERSAM software. The a.s. degradation was assessed by using a first order kinetics reaction. The comparison of the overall risk was done by calculating the "area under curve" and estimating the risk reduction. Considering the IPM system, there was a risk reduction among all references. This was particularly visible on Ref 3 and Ref 5 (71.9% and 57.5%, respectively) where some of the synthetics were replaced by bio-PPPs and anti-drift nozzles were used. The adoption of other RMM also resulted in an overall risk reduction: 6.8% (Ref 1), 26.4% (Ref 2), and 34.6% (Ref 4). However, despite these values and the efficiency of mitigation measures, none of the references tested fell below the trigger value during most of the application period. The RMM, mainly the replacement of synthetic PPPs by bio-PPPs, resulted in a decrease of the overall risk values, showing their significant contribution to reduce the reliance of crop production on chemical PPPs.

#### **6.07.P Getting the Soil Loop Rolling: Ecotoxicology, Risk Prediction, Monitoring and Back**

##### **6.07.P-Mo372 Application of a Novel Smartphone-Based Digital Image Colorimetry Technique for the Assessment of Soil Salinity**

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Salinization is one of the largest threats facing soils globally as a result of rising sea levels and the use of saline groundwater for crop irrigation. Saline soils have been identified in over 100 countries worldwide, affecting over 1 billion hectares of land. Understanding the scale and extent of soil salinity is essential to enable farmers and land managers to make appropriate decisions about how to effectively apply practices which can help to reduce the impact of problems associated with saline soils. However, soil salinity analysis methods are frequently prohibitively expensive or time consuming, therefore access to low-cost, simple, fast and robust measurement techniques is desirable to help inform land-management approaches and to provide accessible analysis techniques to all practitioners, without cost being a barrier.

In this project, a novel smartphone-based Digital Image Colorimetry (DIC) technique using colorimetric chloride test strips has been developed and compared to other portable and lab-based techniques for the assessment of soil salinity. A survey of a coastal saltmarsh and neighbouring farmland was conducted at the RSPB Mersehead Nature Reserve in Southwest Scotland. The salinity of 70 soil samples was analysed by the new DIC method, as well as 3 other portable and 2 lab-based methods. Comparisons were drawn between the methods in terms of practicality and ease of use, cost effectiveness, accuracy, and precision of measurement. Results presented show that the new DIC method compares very favourably with standard analytical methods for soil salinity, with a correlation coefficient of 0.98 ( $p < 0.001$ ) and 0.96 ( $p < 0.001$ ) with chloride meter and conductivity methods, respectively. The DIC method also performed better than a portable soil conductivity sensor. An application of the DIC method, and other methods evaluated, is demonstrated by using geostatistical analysis (Kriging) to estimate soil salinity across the ~7km<sup>2</sup> Mersehead site, highlighting significant variation in salinity across the area.

Each technique evaluated had specific benefits, such as accuracy, precision, selectivity or speed, however the low cost, portability and lack of specialist equipment required for the DIC technique makes it suitable for use in agriculture, citizen science or conservation settings where resources may be limited. The example presented demonstrates how smartphones may be used successfully to broaden access to environmental analysis techniques.

#### **6.07.P-Mo373 Assessment of Biomagnification Potentials for a Suite of Per- and Poly-fluoro Alkyl Substances (PFAS) in a Soil-Plant-Mammal Model**

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Per/polyfluoroalkyl substances (PFASs) have been linked to accumulation in soil, terrestrial plants and mammals in previous studies with the potential for biomagnification in terrestrial food webs. We investigated the uptake of a suite of PFAS compounds in Timothy grass (*Phleum pratense*) and kale (*Brassica oleracea*) from soil to estimate the biomagnification potential in a soil-plant-rabbit food web. Plants were grown in a modified Organisation for Economic Co-operation and Development (OECD) constructed soil amended with 18 PFAS compounds to generate bioconcentration factors (BCF). The shoot BCFs increased as the C chain length decreased except for Perfluorobutanoic acid (PFBA) and were greater for kale than for Timothy grass. PFBA had the greatest BCF in plant shoots. Root BCFs generally increased with C chain length except for PFBA. PFAS compounds with shoot BCF values  $\geq 2$  were selected for the soil-plant-rabbit biomagnification study. For the biomagnification study, kale plants were grown in an environmentally controlled greenhouse in a modified OECD constructed soil amended with 0.01 mg/kg each of 11 PFAS compounds for 56 days. Kale leaves were harvested from mature plants. Certified contaminant-screened Timothy hay was purchased and amended via atomizer with PFAS compounds at concentrations achieved in the BCF studies. Plant material was fed to Dutch-belted rabbits weekly for a 28-day uptake phase and 28-day elimination phase. Accumulation of the 11 compounds in soil, plants and rabbits were analytically determined. Ecologically relevant data developed in this research will fill several knowledge gaps regarding biomagnification potential for PFAS compounds released into the terrestrial environment.

#### **6.07.P-Mo374 Relative Toxicities of PFAS-Free Replacements of Aqueous Film-Forming Foams for Soil Invertebrates**

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Legacy aqueous film-forming foam (AFFF) formulations containing per- and polyfluoroalkyl substances (PFAS) have been linked to accumulation in soil invertebrates and terrestrial plants in previous studies with the potential for biomagnification in terrestrial food-webs. Alternative PFAS-free surfactant formulations are being developed and evaluated for their ability to meet current US Department of Defense performance requirements. The relative toxicities of PFAS-free AFFF-alternatives, as compared with legacy AFFF formulations are not known. We have developed ecotoxicological data for seven candidate PFAS-free AFFF formulations and a legacy AFFF formulation by determining reproduction toxicity for soil invertebrates collembolan *Folsomia candida*, earthworm *Eisenia andrei*, and potworm *Enchytraeus crypticus*. Test species were exposed in separate studies to each formulation in a natural soil, Sassafras sandy loam, which has characteristics (low clay and organic matter content) expected to support high bioavailability for these materials. Toxicity data derived from this project will be used to develop Soil Ecotoxicological Risk Factors (SERF) to assess which PFAS-free AFFF formulations would exhibit lesser environmental toxicity, while meeting the current performance requirements.

#### **6.07.P-Mo375 MICROSOIL – Investigation of Alternative Test Methods to Correctly Assess the Impact of Plant Protection Products, Biocides and Pharmaceuticals on Soil Microorganisms**

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For the risk assessment of plant protection products (PPP) potential effects on soil microorganisms are addressed by investigating if N-transformation (OECD 216), an important soil function, may be affected. However, by focusing on only one central function of the microbial community, other effects might be neglected.

To determine potential other effects of PPP on functional microbial activity, one objective of the project MIROSOIL is to investigate the sensitivity of three alternative test methods, i.e. substrate induced respiration (MicroResp<sup>TM</sup>), measurement of enzymatic activities (ISO 20130) and effects on ammonium oxidizing bacteria (ISO 15685, AOB). To gather information about the sensitivity of these methods, six model substances were investigated (fungicides: tebuconazole, pyraclostrobin, propamocarb; herbicide: ethufomesate; antibiotic: tiamulin hydrogen fumarate; biocide: DDAC). For sensitivity assessment, results are compared to OECD 216 data.

Up to now, tests were performed in the sandy soils LUFA 2.1 and RefeSol 04A. Three to four nominal test concentrations, e.g. 1x, 5x and 10x of the intended application rate, were tested. Measurements for each test method were performed after 14 and 28 days. If effects above 25% occurred after 28 days, the test duration was extended up to 84 days.

MicroResp<sup>TM</sup> indicated in general a low sensitivity, substance-independently. The group of AOB was strongest inhibited by the test substances. Especially for DDAC (LUFA 2.1 test results), concentration related effects > 25% were determined after 28 days of exposure. For ethofumesate in Refosol 04A, the extracellular enzymatic activities, e.g. phosphatase, altered over the exposure period between stimulation and inhibition. In general, effects > 25% occurred on specific time points, followed by recovery of the respective enzyme activity on the following measurements.

Upcoming experiments with RefeSol 02A will help to identify potential influence of soil properties to the ecotoxicological results. To encounter the impact on the group of fungi, arbuscular mycorrhiza (ISO 10832, *Funneliformis mosseae*) will be investigated under substance exposure. These assessment results of the different soil organisms referring to functional diversity will be complemented by ARISA (Automated Approach for Ribosomal Intergenic Spacer Analysis) structural analysis and presented at the conference.

#### **6.07.P-Mo376 The Influence of Soil Organic Matter Content on the Toxicity of Pesticides to the Springtail *Folsomia candida***

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In the environmental risk assessment (ERA) of lipophilic pesticides (Log Kow > 2), an assessment factor (AF) of two is applied to toxicity endpoints to compensate for differences in soil properties between artificially constructed soils and agricultural soils. This AF is based on earthworm toxicity tests focusing on mortality, and therefore its applicability to other soil invertebrates and toxicity endpoints is questionable. Hence, a better understanding of how soil organic matter (SOM) content influences pesticide toxicity endpoints of other soil invertebrates is needed to improve the ecological relevance of ERA approaches. In the current study, the influence of SOM content on pesticide toxicity to springtails was investigated. Standardized reproduction toxicity tests were performed using the springtail *Folsomia candida*. Animals were exposed to five persistent pesticides with a range of lipophilic properties in four soils (three artificial soils, one natural soil) with different SOM contents, using survival and reproduction as endpoints. The data were used to determine LC<sub>50</sub>, EC<sub>50</sub>, and NOEC values, and the relationships between toxicity endpoints and SOM content were analysed through linear regression analysis. The results show that, for all tested pesticides, the acute and chronic toxicity was lower in soils with higher SOM content. For chlorpyrifos (Log Kow = 4.70), SOM influenced lethal effects more than sub-lethal effects, while for lindane, cyproconazole, and imidacloprid (Log Kow = 3.50, 3.09, and 0.57, respectively) the toxicity-SOM relationships were similar for both toxicity endpoints. For carbendazim (Log Kow = 1.48), no dose-response curves could be constructed. Overall, the results indicate that toxicity-SOM relationships differ between pesticides, which implies that having a single AF for all lipophilic pesticides may not be fully appropriate. Additionally, the current research shows that SOM content also influences the toxicity of pesticides which are considered non-lipophilic under current legislation.

#### **6.07.P-Mo377 The Power of Soil Water in Ecotoxicological Testing with Springtails Following OECD 232 Using Natural Soils**

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In 2017, the European Food Safety Authority (EFSA) issued a scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, stating that a standardised arable soil closer to the scenarios in the exposure assessment would be preferred over the OECD artificial soil. As most agricultural soils do lack of extreme properties for practical and fertility reasons, factors influencing a successful control performance in reproduction testing with *Folsomia candida* are less soil properties like texture or pH value but more the soil water availability. If the matric potential (soil water tension) exceeds pF 2.18 (-150 hPa), reproduction of *F. candida* is completely stopped and will only restart, when soil moisture conditions are wetter. This means, when investigating the toxicity of substances on in-soil organisms in natural soils not only soil properties like organic matter or species specific sensitivity can influence the results, but also the soil moisture status. Beside biotic stress due to different water availability, the exposure scenario for in-soil organisms is also influenced by precipitation and drought events, due to differences in sorption behaviour and therefore bioavailability of a substance.

To meet the demand of alternative natural soils for standardised tests, a sensitivity testing was performed with four reference soils that are representative for agricultural use for species *F. candida* according to OECD 232. The toxicity of an appropriate reference substance on *F. candida* exposed in natural soils was tested in comparison with OECD artificial soil. Additionally, a co-stressor of differences in the soil moisture status was included.

The results show, that the usage of a wide range of agricultural soils with diverse properties are suitable for laboratory testing according to OECD 232. A key factor for a successful testing with diverse natural soils is an appropriate actual soil water availability that can be ensured with pF values as a measure for soil moisture instead of the maximum water holding capacity. In general, the exposure scenario and toxicity of plant protection products for in-soil organisms in natural agro-ecosystems is influenced by the fate of a substance and their bioavailability in the habitat soil. This bioavailability is influenced by a multitude of factors and the results presented will reveal the role of the soil water status and soil sorption properties.

#### **6.07.P-Mo378 Use of Natural Soils as Intermediate-tier for Non-target Soil Organisms Risk Assessment of Plant Protection Products at EU Level**

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According to the European regulation (EC) No 1107/2009, an ecotoxicological regulatory risk assessment (RA) for Plant Protection Products (PPP) typically follows a tiered approach, based on a large range of organisms tests. Regarding non-target soil meso- and macroorganisms the first-tier RA is based upon chronic toxicity data from laboratory studies using standard artificial substrates. These laboratory studies are performed in controlled conditions following recommendations of dedicated guidelines (e.g.: OECD, ISO). Toxicity values are then compared to the predicted environmental concentrations in soil. In case of

unacceptable risk, field studies can be performed to assess the potential effects on soil organisms communities in realistic conditions. However, to date no other refinement options are formally available.

The use of artificial soils for Tier 1 RA has many benefits, as these soils are easy to use and allow good reproducibility between laboratories. However, due to their high organic matter content, clay composition and soil texture, these soils may not be representative of agricultural soils / natural soils and potentially drive to confounding effects that bring uncertainty in a RA context.

The guidance on the risk assessment of PPP for soil organisms is going to be revised in the upcoming years. Meanwhile, an EFSA scientific opinion (2017) proposed a new framework for risk assessment of soil organisms. This opinion also identified intermediate tier approaches as a focus for future research that need development, standardized test designs and further guidance. Among all recommendations provided, it is encouraged to identify and use natural agricultural soils in standard laboratory testing to characterize parameters potentially modulating toxicity of PPP in order to better extrapolate toxicity data from the laboratory to field situation.

Through a scientific literature analysis, the aim of this poster is to (i) identify relevant and reliable toxicity data using natural soils for standard soil organisms (e.g. *Eisenia fetida/andrei*; *Folsomia candida* and *Hypoaspis aculeifer*) using natural soils; (ii) compare these toxicity endpoints to the ones currently used in the EU risk assessment for PPP and determine which parameters mostly influence toxicity results; (iii) identify if the outcome of the RA would be different and propose recommendations for further research and development to better address non-target soil organisms RA in the upcoming years.

#### **6.07.P-Mo379 Intermediate-Tier Approach for In-Soil Meso- And Macroinvertebrates Risk Assessment for Plant Protection Products Registration: Species Sensitivity Distributions (SSD), Application and Limits**

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In the frame of the European regulation (EC) No 1107/2009, a regulatory risk assessment (RA) is performed for all Plant Protection Products (PPP). According to this regulation, a large range of organisms is required and current guidances on ecotoxicology RA usually followed a tiered approach. For soil organisms when exposure cannot be excluded, a first-tier RA is required based on chronic toxicity data from laboratory tests in controlled conditions (*i.e.*, following recommendations of dedicated guidelines or academic standards) using artificial soils. Some refinements using natural soils are proposed. These laboratory studies are conducted on three species groups: earthworms, collembola, and mites, represented by three standard species, *i.e.*, *Eisenia spp.*, *Folsomia spp.*, and *Hypoaspis aculeifer*, respectively. Ratio between toxicity value and predicted environmental concentration in soil is compared to the threshold value for acceptability of effects under intended use conditions. In case of unacceptable risk after first-tier RA, higher-tier assessment with field studies can be performed to assess the potential effects on invertebrate soil communities in more realistic conditions. To date, no other refinement options are available in the current SANCO guidance document (2002). However, in the EFSA opinion (2017), a new framework with revised community protection goals was proposed including intermediate-tier approaches, which still need development, standardized test designs and further guidance.

One of these approaches is to address the sensitivity of species belonging to one or more taxonomic groups using the Species Sensitivity Distributions (SSD). This method is based on laboratory data to estimate the Hazardous Concentration affecting 5% of the tested species (HC<sub>5</sub>). In many publications, SSD are built based on a combination of different parameters: *e.g.*, toxicity data from active substance and/or PPP; different types of substrates; different endpoints measured; etc. However, their reliability remains questionable for RA purposes.

This poster seeks to (i) identify toxicity data available in scientific literature to perform an intermediate-tier approach with a proof-of-concept method conducted on various active substances (ii) identify technical limitations and gaps (iii) propose recommendations to build more reliable SSD concerning the soil organisms RA (iv) suggest development to further address the soil meso- and macrofauna RA in the upcoming years.

#### **6.07.P-Mo380 Further Testing of the Fungicide Fluazinam – Effects on Three Non-Target Species**

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Plant Protection Products (PPP) are extensively applied chemicals, to protect crops against pests and are one of the primary pillars of high production modern age agriculture. However, PPPs applied in agriculture can have unintended toxic effects on non-target organisms. As a result, modern agriculture represents a constant struggle between chemical driven high yield of edible crops, and the challenge of sustainable production.

Due to their toxic nature, PPP are subject to a risk assessment before they are authorized for the use in agriculture. In Europe, the risk assessment is performed by the European Food and Safety Authority (EFSA) and for soil, it is mostly based on chronic effects of single products on few standard surrogate species.

In this study, we chose to focus on the fungicide fluazinam, because its authorization is based on limited ecotoxicological data. First, reproduction effects for the springtail *Folsomia candida* are expressed as unbound values. Secondly, laboratory results suggested high toxicity on the reproduction of the earthworm *Eisenia fetida*. However, field studies did not show any adverse effects. These contradictory results lead to some controversy concerning the risk of fluazinam application. As such, the risk was considered as acceptable by European and Canadian regulatory agencies but not acceptable by Norway, which decided to ban the substance.

To generate further data on the ecotoxicity of fluazinam, we performed reproduction tests on three terrestrial species *Folsomia candida*, *Folsomia fimetaria* and *Enchytraeus crypticus*. Testing was carried out, under standard laboratory conditions, using LUFA 2.2 soil as testing substrate and following standard OECD guidelines. Additionally, test concentrations were measured at the start and at the end of the test using an LC-MS/MS system. Preliminary results indicate a high sensitivity of *E. crypticus* and *F. fimetaria*, with effects detected below the lowest relevant measured environmental concentration. The results of this study provide further evidence that fluazinam might present an unacceptable risk for soil organisms and its use should be re-evaluated to establish a safe application pattern. The study also demonstrates that other non-target species than the ones routinely tested in the authorization procedure like *F. fimetaria* and *E. crypticus* can have a higher sensitivity to certain PPP.

#### **6.07.P-Mo381 Use of natural soils to refine the risk to earthworms in India – A Case Study**

**Stefan Kimmel**, Corteva Agriscience, Germany

At present there is no specific guidance for intermediate steps to refine the risk assessment for earthworms. Currently, guidance includes only 2 tiers: Tier 1 laboratory studies (OECD 222) and Tier 2 field studies if a potential risk is triggered.

At Tier 1 the assessment of toxicity to earthworms performed in an artificial soil substrate. This substrate does not necessarily reflect the behavior of chemicals in the range of natural soils found in the environment. This presentation describes an intermediate refinement using representative natural soils from India. To refine the hazard assessment of a fungicide, the potential adverse effects in a range of natural soils was tested to increase the environmental realism and relevance of the studies.

Representative batches of natural soils from across India were selected based on different soil textures and classes. Reproductive performance of the earthworms under a range of test concentrations in the natural soils was compared to untreated controls as well as artificial soil according to modified OECD 222 to enable more realistic evaluation and standardization of the tested natural soils. The results will be presented alongside how this approach might be incorporated within existing risk assessment frameworks for soil organisms.

This case study demonstrates the value of tailor-made study designs to develop appropriate approaches for refined risk assessment of soil organisms.

#### **6.07.P-Mo382 The Effect of the Estimation of Different Hypotheses Test with Different Transformation on the Estimated Endpoint - Consequences of Wrongly Used Tests on an Endpoint and its Reliability**

**Thomas Gräff**, Sabine Duquesne, Pia Kotschik, Klaus Swarowsky, Rene Sahm and Silvia Pieper, German Environment Agency, Germany

In higher tier test systems, natural assemblages are tested experimentally and exposed to chemicals to understand their effects on communities representative of field situations. For terrestrial higher tier studies, the regulatory endpoint of interest is the NOEC. For aquatic mesocosm studies, it is also the NOEC and as well the NOEC that are the relevant endpoints; they enable to apply the threshold and the recovery options, respectively. Due to the high diversity of natural communities, data from higher tier test systems always present a high variance and low numbers of replicates. The endpoint estimation is based on multiple hypotheses tests and the test with the highest statistical power should be selected. For both types of test systems, terrestrial field experiments or aquatic mesocosm experiments, abundance data sets have to be analysed. These data sets are count data and follow the Poisson distribution which is skewed to the right. Parametric tests of the t-test family show a much higher power compared to unparametric tests like Jonckheer- Terpstra or U- test. The preconditions for these parametric tests are normal distribution and/or variance homogeneity. In case of William's test, additionally a linear trend is needed. For aquatic experiments, it is quite common to transform the data with the natural logarithm procedure  $\ln(a \cdot X + 1)$ . In most cases, those preconditions are not proven after the transformation, i.e. it is not checked if the transformed data set fulfills the criteria of the specific statistical test. For terrestrial higher tier tests, data transformation is not usual but also sometimes applied. In these cases, unparametric tests like the U-test, showing a very low power, are used for effect determination. For both types of studies, other statistical tests showing higher statistical power without dependency on data transformation are available. Possible alternatives for parametric tests analysing count data are GLM and GLMM with internal error correction based on Poisson or quasi-Poisson distribution in combination with Dunnett test or CPCAT.

In the present study, several data sets of terrestrial and aquatic higher tier test systems were analysed with different hypotheses tests to estimate the NOEC. Preconditions were also checked, if required. The tests delivering the most protective results under specific conditions were analysed. Different transformation methods were applied, when specific preconditions were required.

#### **6.07.P-Mo383 Alternatives for CP-CAT Test to Evaluate Soil Organism Field Trials**

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The draft OECD guidance (OECD, 2021) recommend the statistical evaluation of species abundances less than five by means of the CP-CAT test (Lehmann, 2016). The rationale for this choice was: "The theoretical distribution assumption of earthworm abundance field test data follows a Poisson model". The CP-CAT test is a combination of 1) Marcus' closed testing procedure in a complete family version for unrestricted comparisons against a control group and 2) a permutation version of the likelihood ratio test for the difference of Poisson means. NOEC estimates are appropriate for small sample sizes, as the proposed  $n=6$  and strict Poisson distributed variable. But the above rationale violates the Box' principle 'All models are wrong, some are useful' by focusing on exactly one theoretical distribution with the strange condition 'variance=mean'. The sum of Poisson variables follows the Poisson distribution when they are independent, which is already violated by averaging the sub-sampling trap counts for a field plot and particularly for the aggregation into higher taxonomic levels. Even empirically over-dispersion and particularly severe over-dispersion were observed in historical control data for selected species in Fig. 24 of the UBA report (UBA, 2020).

Simulations reveal both violation of the alpha level and sub-optimal power when applying CP-CAT test to under/over-dispersed data. Moreover, the current CP-CAT test is numerically only available in a two-sided version, confidence limits are not available, random effect modeling of sub-samples is not available and adjustment against baseline covariate is not possible. Therefore, alternatives were selected that are robust against various violations of the theoretical Poisson distribution, particularly different dispersion parameters in the multiple concentration groups. Candidate tests include a non-parametric Dunnett-type test based on global ranking relative effect size (Konietschke, 2012), the most likely count transformation model (Siegfried, 2020) and generalized estimation equations using variance-sandwich estimators for clustered count data.

#### **6.07.P-Mo384 An Alternative for OECD Decision Tree Approach to Evaluate Soil Organism Field Trials**

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In the draft new OECD guideline for the earthworm field testing (OECD, 2021) a decision tree approach is proposed for the evaluation of soil organism field trial in Fig. 2 (page 18). The first branch divided into rare abundance data or continuous as well as abundance data when no. taxa > 5. The latter is relevant for the majority of raw the data, namely abundances > 5 (and their aggregations to upper taxonomic level) and continuous endpoints like biomass on its various classification levels.

After first branching, the next two branches propose pre-tests on normal distribution (e.g., Shapiro-Wilks test) and homogeneity of variances (e.g., Levene test) (Fig. 2). Based on these decisions an appropriate test out of the method list (Williams or Dunnett, Bonferroni-Welch-t tests, stepdown JT test, Bonferroni U-test, Bonferroni median test) should be selected. Using such pre-tests to decide between proper parametric and non-parametric test is not appropriate and can lead to misleading conclusions. Alternative robust tests without the need of all pre-tests are proposed: I) robust against variance heterogeneity in balanced and unbalanced designs, namely using sandwich variance estimators (Herberich, 2010) for multiple contrast test of Dunnett, Williams and Dunnett-Williams-type (Jaki, 2013), II) robust against violation of normal distribution, particularly count data with not too tiny counts, namely robust parametric, non-parametric and most likely transformed multiple contrast tests. A list of compatible tests is proposed (in difference to Fig. 2 list). A tiny slice of an extensive simulation study is presented.

A real data example (a soil organism field study, Ernst, 2022) is used, to demonstrate these alternative approaches by means of two one-sided 95% confidence limits derived based on using the plot as randomized unit (linear model) and using the subplot within the plots (linear mixed effect model). These intervals are plotted for consecutive monitoring times to interpret species-specific decrease and their possible recovery afterwards.

In summary, recommendations for a routine analysis using CRAN R packages are developed, and an outlook on the analysis of abundance data with very low count numbers is given.

#### **6.07.P-Mo385 Design and Evaluation of Ecotoxicological Field Studies with Soil Organisms (Collembola) for the Registration of Plant Protection Products (PPP) in the EU**

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For the registration of PPPs in the EU, ecotoxicological risk assessments (RA) for soil organisms are required. The tiered approach starts with comparing calculated environmental concentrations and ecotoxicological endpoints from laboratory studies. In case of failure, a higher tier RA can be conducted, which regularly results in field studies, focusing on recovery of non-target soil organisms within one year after application of the respective product. As shown in a previous impact assessment, the new EFSA exposure framework for calculation of environmental concentrations increases the number of compounds failing the first tier RA, i.e., requiring field studies.

However, no official OECD Test Guidelines have been adopted for such field studies; ISO guideline 11268-3 adapted with literature data is used to conduct field studies on earthworms. Collembola field studies are generally conducted following this guidance, yet there is a lack of guidance for evaluation and interpretation of results. High natural variability of data may lead to discussions on biological relevance and statistical significance, with a preprogrammed high uncertainty on the outcome from regulatory perspective.

We have analysed and reviewed historical control data to investigate natural variability in Collembola populations in farmland, between studies and over time. Species abundance for different taxa and sampling points shows the variability in abundance for studies conducted in a similar agricultural environment. The results shall support data interpretation of field studies and help to distinguish between natural variability and biologically relevant test item effects. Available and new statistical methods (including CPCAT, GLMMs and power analysis) to detect potential treatment effects are evaluated, and suitable approaches for statistical evaluation of abundance data are proposed.

Our results can support the development of a future OECD test guideline for soil mesofauna field studies and help with data interpretation. However, with regards to the high complexity of the data and difficulties in interpretation, a full field study should be the last step in the RA and not directly following Tier 1 studies. Expecting that more compounds will fail the first tier RA, intermediate tier testing is required to clearly distinguish between low-risk compounds and compounds that potentially pose a risk.

#### **6.07.P-Mo386 Regulatory Field Studies With Soil Mesofauna – On The Way to a Guidance for Harmonized Assessment of Pesticide Impacts in the Field**

*Gesa Amelung, Pia Kotschik, Thomas Gräff, Klaus Swarowsky and Silvia Pieper, German Environment Agency, Germany*

If the tier 1 pesticide risk assessment for soil organisms indicates an unacceptable risk, current data requirements ((EU) No

283/2013 and 283/2013) give the option to perform higher tier studies on soil mesofauna. Due to practical reasons, field studies with soil mesofauna usually focus on natural collembola and or mite communities. However, no official guidance is available for such studies up to now. This results in high uncertainties and inconsistencies in the conduction and evaluation of respective studies. Currently, European Member States are discussing the development of a respective guideline. To contribute to this process, we evaluated field trials with soil mesofauna submitted to authorities during active substance and plant protection product evaluation. The analysis aimed to gain an understanding on how relevant effects on assessed endpoints can be determined reliably. Statistical analyses helped to evaluate whether endpoints in aggregated form or those related to single species were more relevant to identify effects of the tested substances. Data was retrieved from studies on soil natural mesofauna communities, usually exposed to pesticides under field conditions for 12 months. In case of very persistent test substances, studies were prolonged in order to cover the possible effect duration. We investigated the variability of determined soil mesofauna species versus grouped endpoints additionally to the determination of the statistical power of the studies. Poisson distributed data were analysed via Closure Principle Computational Approach Test (CPCAT) in order to identify statistically significant deviations in responses of soil mesofauna in treated plots compared to control plots. Additional statistical test methods (e.g. Dunnett or William test) were performed depending on the data quality. Analyzing poisson distributed data with CPCAT enabled to detect significant effects at lower concentrations than common statistical methods, which are less suitable for poisson distributed data. Analyses of aggregated endpoints was based on higher individual densities and was often statistically more robust – but in some cases the impact at species level was blurred. Discussion of the biological relevance of the statistically significant difference is important as well as of non-significant effects at large effect size.

#### **6.07.P-Mo387 Software Development for Soil Ecological Risk Assessment in Korea**

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The International Standard Organization (ISO) recently suggested an ecological risk assessment strategy for contaminated sites, so called the TRIAD approach that includes three lines of evidence such as chemical (Chem-LoE), ecotoxicity (Ecotox-LoE), and ecology (Eco-LoE). The three LoE are calculated each as a 0-1 number and ultimately integrated to obtain the final integrated risk (IR) for the contaminated site. Every tiered work has been conducted by hands or using excel worksheets. There is no computer program to automatically calculate the TRIAD approach to contaminated sites. This study constructed a computer program, called KERAS (Korean ecological soil risk assessment program) to include the three tiered approaches. KERAS begins the first two main gates, “Preliminary survey” (PE) and “Ecological risk assessment” (ERA). PE was ready to decide whether a contaminated site should be subject to a further ERA or not, given Korean government situations. PE includes program pages to collect contamination information such as contaminants, concentrations, land uses, ecological and environmental indices. PE decides “further ERA” if the site is located within ecologically sensitive area. PE decides “further ERA” if the site is located within an ecologically sensitive area. If PE decides “further ERA is needed”, an appropriate soil sample size can be computed by statistical power through the EP survey preparation page. If PE decides “further ERA is needed”, an appropriate soil sample size can be calculated by statistical power through the investigation preparation page of PE. ERA includes input pages of each site investigation data for chemical, ecological and ecotoxicity areas. These inputs were automatically calculated to produce each chemistry, ecological and ecological risk, and these three risks can be integrated into the site’s IR. KERAS is also able to display graphically Chem-LoE, Ecotox-LoE, Eco-LoE and IR on each site.

#### **6.07.P-Mo388 Prioritisation of Tomorrow’s Pollutants in Soils**

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Soils provide 95% of our food and inhibit an enormous number of living organisms. However, soils under multiple uses – half of Germany’s land is used for agricultural production, which is treated with pesticides to ensure good growth of cultivated crops. However, chemical inputs into soils may cause threat on soil functions like soil fertility, harm in-soil organisms and thus threaten our food security and human health. The number of registered and used substances used as plant protection products in the EU and Germany is high and their impact on soils is hardly monitored. Usually, monitoring activities only focus on well-known substances, such as heavy metals. Recent monitoring studies show several residues of pesticides in soil samples. Whereby healthy soils are one goal described within the EU soil strategy as well as in the Sustainable Development Goals of the United Nations, a definition of a healthy soil is missing. However, proposals for the definition of soil health include also indicators for chemical pollution.

Guidance values for chemical pollution are urgently needed as soil pollution by chemicals may disturb soil functions, harm in-soil organisms, leach into groundwater, may be taken-up by plants serving as food source for humans and may also be taken up by humans directly. Therefore, it is important to get a deeper understanding of chemicals in soil, released via air or directly via the application of biosolids, fertilisers, plant-protection products, etc. The aim of this work is to identify candidates of organic substances used as plant protection products most harmful for in-soil organisms by a prioritisation concept. Here, we present the selection of relevant parameters describing potential harmful properties of active substances, based on their physical and chemical properties as well as ecotoxicological profiles.

Moreover, we introduce the results of our first theoretical approach based on a simple mathematical model with data available from various data bases. In the course of the European Soil Strategy and the revision of German Soil Legislation this concept is expected to be helpful in providing new insights into soil pollution and requirements for monitoring and regulation.

#### **6.07.P-Mo389 Inventory Analysis of the Contamination of Agricultural Land with Biocides, Pharmaceuticals and Plant Protection Products in Germany**

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Agricultural soils in Germany are exposed to a variety of substances. However, the overall residue contamination situation is unknown. Several substances are applied directly or indirectly to soils and can accumulate there - depending on the respective soil and substance properties. Published studies on agricultural soil pollution in Germany are limited and often restricted to single (often well known) substances or substance groups.

Organisms living in such soils are exposed to numerous chemicals from different uses over a long period of time. However, the risk assessment for active substances of plant protection products (ppp), biocides and pharmaceuticals as well as industrial chemicals under the different European regulations does not consider multiple exposure of substances. In reality, substances under different regulations used within several products reach the soil, e.g.; by spraying series of different ppp, which are applied within one growing season (Knillmann et al, 2021). This has already been identified as one of the major deficits of the single substance assessment in the approval procedures of ppp (Topping et al., 2020; Frische et al., 2018), but is also common practice in the approval procedures of biocides and pharmaceuticals. So far, it is unclear to what extent multiple exposure is relevant in the field situation and whether neglecting the realistic exposure leads to an underestimation of resulting risks in the individual approval procedures of chemicals.

In 2021, a project funded by the German Environment Agency was initiated, with the aim of obtaining a first screening of the pollution level of agricultural soils in Germany together with the investigation of their biological conditions. Knowledge of the current contamination situation of agricultural soils is necessary to check the plausibility of models for regulatory substance approval. Moreover, current assumptions underlying risk assessment schemes for soil organisms can be checked against the situation in the field. An overview of the actual contamination situation in connection with a status survey of the soil organism community can therefore provide a valuable contribution to the further development of risk assessment of pharmaceuticals, biocides, ppp and industrial chemicals and could significantly influence approval decisions in the future. This poster summarizes results of the first sampling period in spring 2022 in terms of chemical residues as well as biological parameters.

#### **6.07.P-Mo390 A Conceptual Framework for Biomonitoring Plant Protection Product Residues in Soil**

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Plant protection products (PPP) are an extensively used tool to protect agricultural crops from pests. To avoid adverse effects of PPPs on non-target organisms, a prospective soil risk assessment is performed prior to their (re-)authorization, focusing on single active ingredients and their products and their effects on single species. Transient effects from which the communities can recover after a short period of time are accepted within the authorisation. So far, neither mixture effects from multiple applications nor potential indirect effects on soil communities are explicitly considered. Therefore, retrospective risk assessment of PPP, especially for long-term effects of PPP residues, to validate these assumptions is needed.

Since soil is a complex matrix, which affects PPPs bioavailability and organism's fitness, the total soil concentrations of chemicals might not directly correlate to effects on soil organisms. To improve the retrospective risk assessment of PPP residues in soil, chemical and biological monitoring should be combined. Current approaches, such as the TRIAD method, designed for site specific risk assessment, can provide an important basis but needs to be adapted to fit a monitoring approach.

For the biomonitoring of PPP residues, a "deconstructed" TRIAD approach is proposed. Since it is not possible to do detailed biomonitoring at all agricultural sites, chemical analysis in combination with generic ecotoxicological risk-based reference values (Soil Guidance Values - SGV) could be used as a screening tool to identify sites potentially at risk. Sites potentially at risk are flagged for more detailed monitoring where, the generic SGV is refined based on specific site properties and ecotoxicological and ecological indicators are applied. Detailed monitoring should be conducted over multiple years (i.e. 5 years), to evaluate the long-term trends of PPP residues and their impact on soil fertility.

Finally, the long-term risk can be evaluated by combining data collected from chemical, ecological and ecotoxicological lines of evidence. The information generated can be used to evaluate and calibrate the bioindicator toolbox as well as the SGV values.

#### **6.07.P-Mo391 Development of an Indicator to Reflect the Specificities of Habitat and Ecosystems in Chemical Risk Assessment**

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Indicators are required to answer the very different needs coming from environmental policy, strategies and aims. This scoping study was conducted with the aim to develop an ecosystem indicator to support the assessment of the progress made in implementing the goals of the European Green Deal. In this scoping study, a methodology was designed reflect the consequences of chemical pressure on terrestrial ecosystems raised by human activities (e.g., farming) taking into account different land use types like specific or protected habitats or intensively used areas such as agricultural landscapes,



In this conceptual study, our leading approach is based on the risk, which can be summarised as a combination of hazard (toxicity) and exposure, but we introduce the specificities of a given habitat for both farmed and natural areas, by applying a protection factor within an indicator. The study focuses on terrestrial ecosystems as an example, and identifies relevant methodologies and datasets related to chemical concentrations, their effects on ecosystems (habitats and species spatial distribution) to produce risk maps for chemicals that could be applied to various ecosystems.

The work conducted highlights the data required for the development of such an indicator and their public availability through a review of existing databases for occurrence and ecotoxicological effects of chemicals as well as habitats descriptors. A case study was developed using data that originate from regular monitoring surveys conducted in Czechia, which is taken as the study area. The indicator is successively applied to different terrestrial habitats based on laboratory data on surrogate plant soil organism species in combination with occurrence data related to residues of active ingredients from PPPs (45 monitoring points mostly in agricultural land). As a background layer, broad ecosystem types available from COPERNICUS High Resolution Layers, or more simplified mapping material, Corine Land Cover (CLC) 2018 Classes as well as The Agricultural Area Mask - a spatial reference layer aiming at achieving the best possible coverage of the Utilized Agricultural Area - were used for biodiversity and habitat information.

The case study illustrates the applicability of the indicator and the need for further development.

## **6.08 Passion for Pollinators: A Decade on the New EFSA Bee Guidance Document and Its Implementation for Pollinator Risk Assessment**

### **6.08.P-Tu400 The New Bee Guidance: Using Equivalence Tests in Higher Tier Studies**

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The proposed use of equivalence test instead of difference tests in the new draft guidance for the risk assessment of PPPs on bees is a sensible approach assuming an adverse effect as seen in the lower tier studies. Using the hypothesis that there is a risk as null hypothesis, places the burden to prove the opposite on the other side.

However, the use of equivalence tests as suggested by EFSA proves to be problematic.

Here, we compare equivalence and difference testing methods using a control dataset of a honeybee field effect study conducted in northern Germany in 2014. Half of the 48 colonies were assigned to a hypothetical test item – group and the colony strength data was analysed using t- tests, a Generalized Linear Mixed Model (GLMM) and the corresponding equivalence tests. The equivalence tests used a 10% equivalence limit and an alpha of 0.2 as suggested by EFSA. The precise calculation of the equivalence tests was designed to the best of our knowledge and understanding of the draft guidance.

The data reflects the natural variability of honeybee colonies, with initially about 15,000 adult bees. Whilst t-test and GLMM confirmed that 24+24 colonies are sufficient to show “no adverse effect”, the equivalence tests of t-test and GLMM were not able to reject the null hypothesis and classified at least some of the assessments as “high risk”, indicating a power that was too low. 24+24 colonies in combination with an equivalence limit of 10% are not able to prove that there is a “low risk” for an adverse effect. An increase of colony numbers in the same year increases the area covered by the study. Alternatively, the study could be conducted again in the next year. Both options possibly increase the natural variability and thus not increase the power of the study as intended, making it impossible to carry out studies that can be accepted by authorities.

### **6.08.P-Tu401 Understanding Frequency and Relevance of Non-additive Effects of Pesticide Mixtures to Honey Bees**

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The toxicity of pesticide mixtures to honey bees has been a concern for decades. When assessing effects of mixtures, the pharmacological principle of additivity defines default expected effects. However, deviations from additivity may occur, namely over-additive (synergistic) or under-additive (antagonistic) effects. Frequency and relevance of synergistic effects of pesticide combinations to pollinators is highly debated. Important considerations are how synergy is measured, and what level of deviation from additivity should be considered “synergy”. There is a lack of appropriate criteria for defining relevant deviations from additivity for bees, and a poor understanding of their frequency. To address these gaps, we investigated a large Bayer database of bee acute toxicity data for binary to quaternary insecticide and fungicide combinations. We computed additivity predictions for these combinations and compared them to the experimental values via Model Deviation Ratios. Moreover, we investigated a large dataset of bee toxicity data from the US EPA Knowledge Base to study the intrinsic testing variability and used this information to propose a numerical threshold to define relevant departures from additivity. Our findings suggest that most acute effects of pesticide combinations to bees can be well predicted by concentration addition, and that the prevalence of synergistic effects is low.

### **6.08.P-Tu402 Bees and Pesticides – Landscape Ecotoxicological Perspectives on Exposure, Effects, and Mitigation**

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Agricultural use of approved pesticides results in bee exposure to a mixture of pesticides. However, we know less about 1) how this exposure and related risk vary among species and landscape contexts, 2) what the biological outcomes are for bee health in

agricultural landscapes or 3) if agricultural interventions could be used to mitigate potential effects. To explore these questions, we used two field site systems, one in Sweden (24 sites) and one in California (15 sites), covering gradients in agricultural land, and five bee species varying in foraging traits. We sampled pollen from all species and used the pesticide concentrations, weighed by toxicity and summed, to estimate pesticide-related risk, along with measurements of bee performance. We found that *Apis mellifera*, an extensive forager, experienced the highest pesticide-related risk that was independent of the landscape context. Two more limited foragers, *Bombus terrestris* and *Osmia bicornis*, experienced increasing pesticide risk with increasing agricultural land. However, pesticide risk was correlated among bee species. We also found that whilst flower plantings were a source of pesticide exposure, they increased *Osmia lignaria* nesting and mitigated pesticide-related reductions in *Bombus vosnesenskii* reproduction. These context dependencies bring the use of single model species, fields, and pesticide product-focus in the environmental risk assessment into question, but also possible roads for development.

#### 6.08.T-01 Bee Guidance Document of EFSA, 2023

**Csaba Szentes<sup>1</sup>**, Pauline Iris Adriaanse<sup>2</sup>, Andres Arce<sup>3</sup>, Andreas Focks<sup>4</sup>, Brecht Ingels<sup>5</sup>, Daniela Jölli<sup>6</sup>, Sébastien Lambin<sup>7</sup>, Maj Rundlöf<sup>8</sup>, Dirk Suessenbach<sup>9</sup>, Domenica Auteri<sup>1</sup>, Alessio Ippolito<sup>1</sup>, Jacoba Wassenberg<sup>10</sup>, Laura Padovani<sup>1</sup>, Agnès Rortais<sup>1</sup> and Franco Maria Neri<sup>1</sup>, (1)European Food Safety Authority, Italy, (2)Environmental Risk Assessment, Wageningen Environmental Research (WUR), Netherlands, (3)Ecology and Conservation Science University of Suffolk, United Kingdom, (4)Institute of Mathematics, University of Osnabrueck, Germany, (5)Federal Public Service Health, Food Chain Safety and Environment, Service Plant protection and Fertilising products, Belgium, (6)Austrian Agency for Health and Food Safety (AGES), Austria, (7)French Agency for Food, Environment and Occupational Health & Safety (ANSES), France, (8)Department of Biology, Lund University, Sweden, (9)German Environment Agency (UBA), Section IV 1.3 - Plant Protection Products, Germany, (10)Board for the Authorisation of Plant Protection Products and Biocides, Netherlands

The bee Guidance Document of the European Food Safety Authority (EFSA, 2013) has been reviewed by a Working Group and the new document is expected to be issued in spring 2023. Nearly all aspects, such as the characterisation of the exposure, the characterisation of the hazard, as well as the lower- and higher tier risk assessments have been reviewed and updated applying innovative approaches and the best available knowledge. Specific aspects, such as assessing chemicals prone to time-reinforced toxicity, assessing sublethal effects, predicting the toxicity of mixtures (i.e. pesticide formulations with two active substances) or the risk from metabolites, were also reviewed. The methods, the used data and the data analysis are all transparently reported. The new document offers workable and detailed user-friendly guidance for the necessary risk assessments that fully respect all the agreed elements of the specific protection goals as set by the risk managers.

#### 6.08.T-02 A Stakeholder's View on the Proposed Updated Regulatory Guidance for Bees in Europe: An Industry Perspective

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The European regulation of plant protection products (PPP) requires an evaluation of the risks to human health and the environment before the European Union level approval of active substances and national registration of formulated products (Reg (EU) 1107/2009). The risk to pollinators is evaluated in this context through a dedicated risk assessment guidance document and the European Food Safety Authority (EFSA) published a revised draft guidance for bees in July 2022 which incorporates many new features. The new draft revised guidance proposes a new risk assessment paradigm that enables a direct comparison of the outcome of the risk assessment calculation to the protection goals set by risk managers.

This presentation will review the main changes contained in this new EFSA guidance proposal in the light of the outcome of an impact analysis performed for a range of PPP and uses, and case studies conducted by industry to test the usability and outcomes of applying the new draft proposal to real-life risk assessment situations. The impact analysis was performed for a range of uses of typical PPP across the EU. In the screening assessment 114 active substances/products and 210 uses were included/ At tier I there were 101 active substances/products and 173 uses (spray applications only).

Based on mortality effects the screening and tier I risk assessments in the draft revised EFSA bee guidance (2022) appear to be more discriminatory for herbicides and fungicides than in the previous draft of 2013. The improved pass rate for these low toxicity compounds for honey bee risk may be attributed to more realistic food intake assumptions and a more realistic approach to chronic exposure and risk. However, the opposite appears to be the case for insecticides and the majority of insecticides may require extensive higher tier data packages, including field studies for which the guidance provided in EFSA 2022 is still not prescriptive enough to ensure the notifier can conduct a realistic and valid study and consequently many of the unresolved challenges of EFSA 2013 may still remain. Furthermore, the impact of time reinforced toxicity and sub lethal assessments for low toxicity compounds requires more evaluation using case studies (which will be provided in the presentation).

#### 6.08.T-03 Sublethal Effects in Honey Bee Risk Assessment

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The lower tier risk assessment of plant protection products (PPP) for bees has historically been focused on lethal effects on adult bees and brood. However, a growing body of scientific research has shown the broad array of sublethal effects PPPs can have on bees which has resulted in calls to include sublethal effects in regulatory assessment. The new EFSA guidance document on the risk assessment of plant protection products on bees (EFSA, 2023) addresses this need by incorporating a subset of sublethal effects in the risk assessment for honey bees. While a large array of sublethal effects was found in a narrative review, very few of

these effects had been studied according to harmonized guidelines, and a link to the SPG was not available. The EFSA Bee Working Group decided to focus primarily on a subset of sublethal effects in honey bees, in particular those that very obviously alter bee behaviour. The rationale for this is that large scale behavioural changes may interfere with important tasks, such as foraging, which would be expected to have a strong mechanistic link/relationship to colony strength. In the proposed tiered approach, existing data that is already collected in line with the legal data requirements is used as much as possible. Initially, mortality endpoints are used with an additional trigger to predict the no-effect level on foraging behaviour. In a second step, the behavioural observations in the standard laboratory tests on bees are used to calculate the no-effect level on behaviour. Guidance for specific refinement studies is presented, including laboratory and higher tier experiments.

#### **6.08.T-04 Testing Chemicals for Time-reinforced Toxicity on Honeybees Based on GUTS Modelling**

**Andreas Focks<sup>1</sup>, Alessio Ippolito<sup>2</sup> and Brecht Ingels<sup>3</sup>,** (1)Institute of Mathematics, University of Osnabrueck, Germany, (2)European Food Safety Authority, Italy, (3)Federal Public Service Health, Food Chain Safety and Environment, Service Plant protection and Fertilising products, Belgium

A substance shows time-reinforced toxicity (TRT) when its toxic effects from exposure to low doses for a long period of time are higher compared to effects from exposure to higher doses for a short period of time (i.e. its toxic effects are reinforced by exposure time). This phenomenon was earlier also called ‘accumulative toxicity’. In the environmental risk assessment of chemicals, analyses are limited to studies assessing the effects from fixed exposure durations (acute and chronic). This approach is valid when toxicity is mainly dependent on the dose. However, in case of substances which show time-reinforced toxicity, the impact of low doses may be underestimated if the exposure period tested in the laboratory is shorter than the environmentally relevant length of exposure. In the new bee guidance document of EFSA, appropriate testing of TRT needs to be implemented. This study explores the use of applying the General Unified Threshold models of Survival (GUTS) framework for the assessment of TRT, with special emphasis on comparing the GUTS-RED-SD and -IT models with corresponding data. A number of data sets were analysed, and GUTS-RED-SD and -IT derived LDD<sub>50</sub> values were compared with experimental observations. It turned out, that LDD<sub>50</sub> values calculated directly from data for some substances clearly follow the dynamics of the GUTS-RED-IT model, whereas other substances follow the GUTS-RED-SD model. Some compounds show data in between IT and SD model-derived values. This shows the importance to always use both GUTS models and to compare with experimental data. conclusion, we are showing here the advantages of using a mechanistic model as GUTS in comparison to using statistical or empirical models like Haber’s rule. Using only statistical models for the derivation of LDD<sub>50</sub> values for single time points results in increased uncertainties for later timepoints. Using Haber’s rule for such analyses is scientifically flawed, since under this rule the LDD<sub>50</sub> of every compound would converge to zero, if the exposure duration would be long enough. The GUTS model has the advantage of “learning” TK and TD from all data points of a test at once, which makes the difference to statistical approaches who use only observations for single time points. Also, GUTS is superior concerning filtering between background mortality and chemical-induced mortality. The recent GUTS-based analyses will inform future strategies for discriminating substances with TRT properties.

#### **6.08.P Passion for Pollinators: A Decade on the New EFSA Bee Guidance Document and Its Implementation for Pollinator Risk Assessment**

##### **6.08.P-Tu403 Developments in the Pollinator RA Following the New EFSA GD and ICPPR**

**Helena Crosland<sup>1</sup> and Gabriel Weyman<sup>2</sup>,** (1)Cambridge Environmental Assessments, United Kingdom, (2)Gabe Weyman Consulting, United Kingdom

The 2013 European Food Safety Authority (EFSA) guidance on pollinator risk assessment remains un-Noted (i.e. is not officially sanctioned for use in the EU). In July 2022 the EFSA released a draft updated guidance on the risk of plant protection products to bees for public commenting. This allowed stakeholders to identify and comment on new features of the guidance and how they may impact on the pollinator risk assessment for pesticides. In addition, in October 2022 the 15th International Symposium on the Hazards of Pesticides to Bees (organized by the Bee Protection Group (BPG) of the International Commission for Plant-Pollinator Relationships (ICPPR)) was held in the UK. The revised guidance was a hot topic of the conference, which was attended (amongst others) by members of the European Commission (EC), EFSA, the EFSA Working Group on Bee Guidance Revision (WG); and the Organisation for Economic Co-operation and Development (OECD).

Both the public commenting period and the ICPPR conference have helped to clarify some areas of the pollinator risk assessment; however there remain some significant areas of uncertainty. This poster aims to summarize some key concerns raised in the public commenting period and the updates (if any) provided on these topics at the ICPPR conference. For example, the Time Reinforced Toxicity (TRT) assessment and potential impact of this (particularly for low-toxicity substances); the lack of a calculator tool; the state of currently available test guidelines compared to the requirements in the updated guidance document; and the risk assessment for non-*Apis* species. Generally, from the presentations and discussions at ICPPR, it seems that the commenting period succeeded in raising important areas of concern with EFSA and the WG (who intend to work further on some topics), but that the guidance document is unlikely to receive significant revisions before release (which may or may not occur before this poster is written and presented at SETAC EU 2022). The full impact of the revised guidance document on future pollinator risk assessments remains uncertain. We will summarise the certainties and uncertainties remaining by the time of the SETAC EU 2022 conference.

##### **6.08.P-Tu404 Review of the Lower Tiers of the Succeeding Crop Exposure Scenario in the new Bee Guidance Document of EFSA, 2023**

**Laura Padovani<sup>1</sup>** and **Csaba Szentes<sup>2</sup>**, (1)*Risk Assessment Production - Pesticides, European Food Safety Authority, Italy*, (2)*European Food Safety Authority, Italy*

In the succeeding crop scenario, bees are exposed to pollen and nectar contaminated with residues of a plant protection product (active ingredient and/or metabolites) that are already present in the soil following the treatment of the preceding crop. Residues accumulated in soil from the previous pesticide application might be taken up by the roots of the succeeding annual crop and then translocated via the vascular system to nectar and pollen. This may also happen for the permanent crop next year or for annual crops that are grown twice in a growing season on the same field ("double crops"). In practice, the succeeding crop is relevant when the use of a plant protection product on a primary crop has the potential to lead to the transfer of residues in succeeding crops via uptake from soil due to the characteristics of the compound (persistence and mobility in soil, transport mechanisms in the root zone). In the EFSA Guidance Document on the risk assessment of pesticides on bees (EFSA, 2013), the assessment of the succeeding crop scenario was required for all substances unless the DegT50 (half-life at 20°C and at a soil moisture content at field capacity) is less than 2 days for applications within the same year and 5 days for applications in different years. However, these triggers were later on criticized because almost all substances triggered the need for further assessments. Therefore, the Working Group tasked to review the bee Guidance Document of 2013, has decided to reconsider the lower tiers of the succeeding crop scenario by setting a screening level to identify those substances that would lead to an exposure level that will not cause adverse effects on bees for this scenario. Based on the available data and analysis, it was concluded that for the dietary route of exposure, the succeeding crop scenario cannot be excluded a priori but its relevance should be always considered during the problem formulation. However, for the annual "double crops" and for the permanent crops the following year, it was concluded that this scenario can be excluded based on specific combinations of soil persistence and soil adsorption properties of a substance with toxicity endpoints  $\geq 0.1 \mu\text{g}/\text{bee}$  and at a given application rate.

#### **6.08.P-Tu405 Field Studies on Pesticide Residues to Refine Exposure to Bees: Recommendations in the Bee Guidance Document of EFSA, 2023**

**Laura Padovani<sup>1</sup>**, **Csaba Szentes<sup>2</sup>**, **Pauline Iris Adriaanse<sup>3</sup>**, **Jacoba Wassenberg<sup>4</sup>**, **Maj Rundlöf<sup>5</sup>** and **Alessio Ippolito<sup>2</sup>**, (1)*Risk Assessment Production - Pesticides, European Food Safety Authority, Italy*, (2)*European Food Safety Authority, Italy*, (3)*Environmental Risk Assessment, Wageningen Environmental Research (WUR), Netherlands*, (4)*Board for the Authorisation of Plant Protection Products and Biocides, Netherlands*, (5)*Department of Biology, Lund University, Sweden*

Field studies on pesticides residues in pollen and nectar are used to refine the exposure assessment for bees in the EU regulatory context. However, to date, detailed and comprehensive technical guidance to ensure the practicality, reliability and validity of these studies is lacking. To prevent potential concerns about the defensibility of bee-relevant residue studies during the evaluation of the marketing authorisation, the new EFSA Guidance Document on the risk assessment of plant protection products on bees (EFSA, 2023) includes clear recommendations on exposure refinement for honey bees, specifically on the test principles, the experimental design, the field phase, the analytical phase and the assessment of residues decline in pollen and nectar. In particular, scientific and technical aspects of field studies aimed to measure residues in pollen and/or nectar have been provided to cover the spatial scale (i.e. the minimum number of sites required to refine the exposure assessment and how to select the locations where to conduct the field study), the temporal scale (e.g. the number of samples, the sampling intervals...), the crop selection and the sampling method. In addition, the regulatory community is unequivocally advised on the selection process for the RUD (Residue Unit Dose) values available from the higher tier studies and that can be used in the exposure assessment refinement. Some considerations on the field exposure studies for non-*Apis* bees are also provided.

#### **6.08.P-Tu406 Higher Tier Studies on Pesticide Effects on Bees: Recommendations in the Bee Guidance Document of EFSA, 2023**

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EU legislation requires the application of a plant protection product to have no unacceptable effects on bees. In the new EFSA Guidance Document on the risk assessment of plant protection products on bees (EFSA, 2023), risks to honey bees, bumble bees and solitary bees have to be assessed separately. If concerns cannot be excluded in the lower tier risk assessment, which is based on laboratory effect studies, refinement is possible by studying the effects in a more realistic setting. EFSA (2023) provides a strategy for the three different bee groups that is based on the Specific Protection Goals set by the risk managers. Tests can be performed under confined or full-field conditions. Field tests, with bees freely foraging on a treated crop, are considered the highest tier. Feeding tests, in which spiked food is offered directly inside the colony, and cage tests are also included as less demanding options, albeit for a limited purpose for honey bees. EFSA (2023) includes specific recommendations on the test principles, the experimental design, exposure verification, the starting conditions of the test species, the duration of the tests, the endpoints and the statistical analysis.

#### **6.08.P-Tu407 BEEHAVE - Analysing the Significance of Increased Brood Termination Rate on the Colony Strength in Honey Bees**

**Alexander Singer**, **Matthias A. Becher**, **Johannes Lückmann**, **Oliver Jakoby**, **Marcus Metz** and **Daniel Ruf**, *RIFCON, Germany*  
The brood termination rate (BTR) investigated in OECD GD 75 studies for pesticide risk assessments is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult. However, it is questionable what an increase of the BTR means for the protection of honey bee colonies. The current EFSA Bee Guidance (2013) on pesticide risk assessment, indicates risk to honey bee colonies, if the overwintering colony size in late autumn drops below 5000 bees. Following this guidance,

previous work indicated potential risk to colonies only if high BTRs towards the end of the bee season occurred (e.g. caused by highly brood impacting reference compounds applied in August). In contrast, the updated Specific Protection Goal (SPG) for honey bees in the draft version of the revised EFSA Bee Guidance of July 2022 defines that a reduction in colony size of up to 10% at all time after pesticide application compared to control bee hives is acceptable.

Therefore, we investigated what the BTR means in terms of this novel risk definition (i.e. > 10% colony size reduction). Using the honey bee colony model BEEHAVE, we simulated colony dynamics following an increase in the BTR. Firstly, we estimated natural variability in colony size to understand if a 10% difference to control reliably can be measured in OECD GD 75 test settings. Secondly, we investigated the plasticity of a bee colony and its ability to recover from increased BTRs. Thirdly, we examined in a sensitivity analysis how BTRs of different magnitude and duration affect colony strength relatively to control bee hives. Our results show the consequences of an increased BTR for colony strength relative to control and sheds light on the impact of the suggested new risk definition in the draft guidance.

#### **6.08.P-Tu408 Food Jelly, A Potential Source for Pesticides to Harm Honey Bee Larvae?**

**Silvio Erler**, Julius Kühn-Institute (JKI), Germany

Honey bees are of public interest because of their pollination service, and production of honey, wax, and propolis. Further, they are considered as model organisms in pesticide risk assessment on pollinators. Previous studies have investigated residue analysis in different bee matrices like honey, bee bread or wax, and some in larval food jelly. We summarize the findings dealing with pesticide residues in all larval food jellies and elaborate factors influencing the pesticide occurrences as well as possible harmful risks for the larvae. It was demonstrated that residue analysis were mainly done in royal jelly, while only one study focused on residue detection in worker jelly. Overall 30 out of 176 pesticidal substances could be detected in a range of 0.15 to 3860 ppb. The experimental setup, respectively the exposure scenario, was the major factor influencing the pesticide occurrence. A comparison of the detected doses with available reference values from risk assessment on honey bee larvae, showed no lethal effects for larvae. The literature screening revealed that there are still missing information on pesticide degradation within different honey bee matrices, possible effects on larval physiology, contamination pathways and associated accumulation or dilution factors of contaminants.

#### **6.08.P-Tu409 Bee Sensitivity Derived from Acute Contact Tests Biased by Standardised Protocols?**

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For non *Apis* bees acute contact test results are the only source available for comparing the sensitivity of bees. However, these tests are carried out according to the honeybee test template: i.e., an assessment based on a 48-hour test, regardless of the size of the bee. In addition, the volume that is applied on the bee is standardized to a 1 µL droplet. Only the bumble bee is treated with a 2 µL droplet. This raises the question if this influences the outcome of the test, as relative surface area that is covered in a test strongly depends on the size of the bee. In our experiments two questions were addressed: does the droplet size affect the kinetics of the toxicant? And does the droplet size affect the sensitivity of the bee?

Therefore, an assessment was made on how much surface area is covered by using different droplet sizes and solvents for four species of bees (alfalfa leaf cutting bee, red mason bee, honeybee and bumble bee) and how this affects the observed effects. The tests on the covered surface areas were carried out with different droplet sizes with a coloring agent in either the solvent acetone or Triton X-100, both frequently used in acute contact tests. In addition, effect data were generated for the different droplet sizes and exposure to dimethoate. Effects were interpreted with recently developed Toxicokinetic Toxicodynamic (TKTD) BeeGUTS, allowing to separate the kinetics from the dynamics.

It showed that the sensitivity of the bees expressed as their threshold for effects was not affected by the droplet size. However, the kinetics appears to depend on the droplet size. In other words, the 'effect clock' ticks faster with increasing 'relative' droplet size. So, with the standard tests and an assessment based on the 48 hr. LD50 this would introduce an additional source of bias when testing a standard droplet size on differently sized bees.

#### **6.08.P-Tu410 Improved Test Design for the Evaluation of Semi-Field Studies with Bumblebees (*Bombus terrestris*)**

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The revised EFSA bee guidance document defines new requirements for the testing of bumblebees and solitary bees in higher tier semi-field and field studies. The colony strength or population size, as well as the reproductive output were set as Specific Protection Goal (SPG). However, the effect size could not yet be determined.

Between 2016 and 2017, the icppr non-*Apis* working group developed and tested a methodology for a bumblebee semi-field test which was based on endpoints used in honey bee semi-field studies (ring-test). The varying results showed that test methods and end points cannot easily be adopted from established semi-field tests for honey bees (OECD 75 and OEPP/EPPO 170). In particular, the colony assessment and most importantly the production of new queens could not be controlled sufficiently in the test.

Factors influencing the varying development and queen production could be the variability of commercially available bumblebee hives as well as the strong disturbance of colonies during intensive colony assessments during the field phase of the trial.

IPM Impact and tier3 solutions GmbH developed a test design for semi-field studies which reduces the variability between the colonies by using special maximal standardized R&D hives with the same colony size and age structure. It minimizes the impact on the natural development of the colonies and their reproductive success (forming of new queens) by using minimal invasive assessment methods during assessments as well as additional modification of hives. This semi field test system fulfils the requirement for bumblebee semi-field studies in the revised bee guidance, such as colony strength and provides further reliable parameters.

#### 6.08.P-Tu411 Oomen Studies –How To Find The Right Concentration To Test

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To protect pollinators from unwanted effects from pesticides there is a compulsory risk assessment necessary before a product can be registered for usage.

The risk assessment has a TIERed approach. First risk is calculated with values received in lab studies and default exposure predictions. In a second step exposure can be refined with nectar and pollen residue studies. If there is still risk calculated higher TIER studies can be run. The Oomen feeding study (Oomen et al., 1992, Lueckmann und Schmitzer, 2014, 2019) is the next higher TIER when a risk for brood is identified at lower TIER level, based on data obtained from the lab studies. It is a robust a reliable study design that exposes the brood and nurse bees directly to the pesticide via food.

In the study design bee hives are fed directly with sugar solution that is spiked with a formulated pesticide. In the original publication the proposal for the determination of the testing rate was the concentration in the spray solution recommended for field use. However, this is a very conservative approach and not really a risk refinement. Furthermore, there exist now several publications from regulatory agencies that estimate residue values in nectar and pollen as well as consumption rates of different life stages of honey bees. But there is no guidance from authorities on how to calculate feeding exposure rates and different national authorities might follow different approaches.

Based on information available in the most recent draft pollinator risk assessment guidance document from EFSA a more realistic concentration for feeding concentration for testing should be agreed upon. The treatment concentration should be based on a back calculation from the residue intake (Rint/PEQ) estimated for TIER 2 exposure estimation. In lack of TIER 2 exposure estimation, TIER1 exposure estimation might be considered. It is expected that in most of the cases, the higher of the 90th percentile nectar or pollen concentration should result in a sufficiently high test concentration (i.e. sufficiently high residue intake in the test) (EFSA 2022).

The poster will show a possibility to calculate feeding exposure rates from the guidance document and will give possibilities for refinement of TIER2 studies with available data from exposure studies.

#### 6.08.P-Tu412 Semi-Field Test Design for the Leafcutter Bee *Megachile rotundata*

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Solitary bees came into regulatory focus with the 2014 published draft guidance document for the risk assement of pollinators. Before, only honey bees were tested as a surrogate species. As a consequence, a semi-field test design with the red mason solitary bee *Osmia bicornis* L. was ringtested by the ICPPR non-Apis working group in 2016 and 2017. The result of the ringtests was presented and published by the ICPPR non-Apis working group with a recommendation for a semi-field test design in 2021. With the knowledge on differences in exposure pathways between the solitary bee *Osmia bicornis* and leafcutter bees (e.g. *Megachile rotundata* F.), it is expected that the same plant protection products will impact those species differently. In addition, a higher sensitivity of *Megachile* species to selected plant protection products was estimated.

Since there is no established test design for solitary bee studies with *Megachile* so far, the main objective of the test was the methodological development of a standardised Tier II study semi-field design based on the recommended test design for *Osmia*. In the leafcutter bee study design, bees (*Megachile rotundata* F.) were released as emerged adults in tunnels containing a bee attractive flowering alfalfa (*Medicago sativa* L.) in Spain and were exposed during their reproductive period. The semi-field tunnel study included a water treated control and a reference item (dimethoate) sprayed treatment. After the application of dimethoate, the bees collected all relevant nest and food items from the treated crop and were exposed via contact to the treatet Alfalfa. This included not only pollen and nectar but also treated leaves for nest building. The evaluated endpoints were the establishment of actively nesting females at the nesting units (nest occupation), observations of the flight activity in front of the nesting units and the production of brood cells.

The assessed endpoints were evaluated with respect to their potential for the use in the risk assessment of plant protection products. Results of this semi-field test will be presented and recommendations for the adaptation of the semi-field test design to an additional species will be given.

#### 6.08.P-Tu413 Testing Methods for Honey Bees, Bumblebees and Solitary Bees in the Context of Pesticide Registration

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Pollinators are an important organism group in general but especially in the agricultural landscape. They fulfil an ecological task that has been recognised since a long time. Pollinators belong to different orders of insects but there are also pollinating birds and mammals. However, across all pollinators, honey bees, bumble bees and solitary bees belonging to the order Hymenoptera are the most important group.

Regulatory authorities for Plant Protection Products (pesticides) require an assessment of the impact on the pollinating species. At present there is regulation in the European Union, North America, Brazil and Japan. The European regulation is being updated at

the moment to take into account the most recent scientific developments. Also, expert groups are active in updating the set of testing methods to be used in revised regulatory context. The talk will provide state of the art of actual testing methods and their developments, linked to OECD, ICPPR and EPPO. It will also show endpoints used in the risk assessment. Methods will address the honey bee (*Apis mellifera*) and will illustrate developments for some other species (*Bombus* spp. and selected examples of other solitary bees).

#### **6.08.P-Tu414 Investigating Potential Effects of a Common Fungicide on Honey Bee Colonies Under Field Conditions When Applied According to Best Agricultural Practice in Flowering Oilseed Rape**

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As an effective fungicide against multiple crop diseases and due to its low toxicity, both to adult and larval stages of honey bees, boscalid is used in a variety of crops incl. the flowering period in attractive crops like oilseed rape or almonds. This can lead to multiple potential exposure pathways for honey bees, both short term through contact exposure via overspray as well as medium or long-term via bee food items like pollen and nectar, which are stored as honey and bee bread. Thus, focus on boscalid and its effects on honey bees has increased in the past years. Despite the considerable amount of scientific research on potential effects of boscalid on honeybees, to our knowledge, no study has yet addressed potential effects of boscalid on colonies under realistic agricultural conditions. Therefore, our focus was on potential effects of boscalid, applied as the commercial solo-formulation CANTUS®, under field conditions according to best agricultural practice on flowering oilseed rape. Honey bee colonies were located at the edge of both untreated (UT) and treated (T) test fields for 45 days before being relocated to a monitoring site, where the colonies were overwintered until spring of the following year. Parameters measured were mortality, foraging activity, colony development, residue levels in pollen and nectar as well as overwintering success. While residue analyses confirmed exposure to boscalid in the colonies located at the treated fields, no adverse effects were observed in the T test group compared to the UT test group regarding mortality foraging activity, colony development, as well as overwintering success.

To our knowledge this is the first time that such an extensive study was conducted for a substance other than an insecticide and it confirms the low risk indicated based on standard laboratory studies.

#### **6.08.P-Tu415 The Revised EFSA Bee Guidance in Practice: An Insecticide Case Study**

**Elizabeth Collison<sup>1</sup>, Vincent Kramer<sup>2</sup>, Stefan Kimmel<sup>3</sup> and Ed Pilling<sup>4</sup>, (1)Corteva Agriscience, United Kingdom, (2)Corteva Agriscience, United States, (3)Corteva Agriscience, Germany, (4)Corteva Agriscience, United Kingdom**

The draft revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) was published by the European Food Safety Authority (EFSA) for public consultation in July 2022. The proposed risk assessment methodologies in this revision are substantially different to those in the previous EFSA 2013 guidance document at all tiers and include some new schemes such as sublethal effects and time-reinforced toxicity assessments. Whilst the draft revised document presents some working example calculations for hypothetical substances and sensitivity/impact analyses for a very limited number of scenarios and elements of the proposed scheme, to the best of our knowledge there is as yet no case study for an active substance in which the full scope of the revised guidance is considered. Here, we will present a pollinator risk assessment for an EU representative open field use of the active substance sulfoxaflor according to the revised EFSA guidance published in 2022. This assessment will follow the proposed tiered approach and use existing laboratory and higher tier studies that have previously been submitted at EU level by Corteva Agriscience. We intend to compare the outcomes of the risk assessments conducted in accordance with the 2013 and 2022 EFSA guidance documents and, if relevant, identify any areas of the revised guidance that cannot be addressed based on the currently available data.

#### **6.08.P-Tu416 Bees and Microbial Pest Control Products, Case Studies with *Bacillus thuringiensis* ssp. *aizawai***

**Silvio Erler, Julius Kühn-Institute, Germany**

Microbial pest control products are commonly applied worldwide as alternatives to avoid potential adverse effects of chemical plant protection products. These products are discussed as being highly species-specific, with no or only mild effects on non-target organisms. There is no information on potential interactions between microbial pest control products and bees' response after exposure to combinations or variable environmental conditions. We aimed to evaluate the biosafety of a commercial product containing *Bacillus thuringiensis* ssp. *aizawai* (strain ABTS-1857) using several experiments with honey bees and other bees as non-target organisms.

Our approaches included 1) laboratory chronic exposure to evaluate the survival of adult and larval honey bees, 2) in-hive feeding under field conditions to examine the effect of *B. t.* on brood development and the core gut microbiome of adult honey bees, 3) semi-field colony-feeding to determine contamination levels of *B. t.* spores in various matrices, 4) a field trial with spray application in a bee-attractive crop to estimate potential environmental accumulation and exposure of honey bee colonies, and 5) laboratory exposure assays to evaluate bees after exposure to mixture of different microbials.

Adult bee and larval survival were negatively affected after chronic exposure depending on the tested concentrations; however, pollen feeding to adults promoted survival of treated bees and delay the effects. Under colony conditions, treated colonies showed a higher brood termination rate and a significantly lower normalized abundance of the core gut microbiome in worker bees. *B. t.* spores were detectable in all matrices at different concentrations, decreasing over time under semi-field conditions. High spore levels were present in honey sacs and pollen pellets immediately after application. No spore reduction was seen in stored matrices like nectar and bee bread. A mixture of *B. thuringiensis* ssp. *aizawai* and *B. amyloliquefaciens* was identified to cause reduction in the lethal time compared to the solo-products.

In conclusion, the pest control product containing *B. t. ssp. aizawai* (strain ABTS-1857) showed a negative effect on exposed bees under laboratory as well as field conditions, for instance on colony development and caused dysbiosis of the gut microbiome. However, further field-realistic exposure studies in bee attractive crops are needed to evaluate the potential risk of such products on honey bees.

#### **6.09.P Practical Implementation of the Essential-Use Concept Through Alternatives Assessment and Functional Substitution**

##### **6.09.P-Mo392 Data for Decision-making: Do REACH Applications for Authorisation Provide Sufficient and Relevant Information to Assess the Essentiality of a Use?**

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The European Commission's Chemicals Strategy for Sustainability stipulates investigating the integration of the so-called essential use concept (EUC) into existing EU chemical regulations, such as the REACH Authorisation which manages the continued use of substances of very high concern (SVHCs). The EUC aims to protect consumers, vulnerable groups and the natural environment from the most harmful chemicals by deciding if their continued use is necessary for health, safety or critical for the functioning of society, given there are no alternatives available that are acceptable from the standpoint of environment and health. A recent analysis by Figuière et al. (2022, under review) has shown that the EUC could be potentially integrated into the Authorisation procedure of the REACH Regulation because sufficient and relevant data are already demanded from companies that apply for authorisation of continue using an SVHC. These data comprise impact assessments on health, social aspects, the (wider) economy and society as a whole, given the guidance of the European Chemicals Agency (ECHA) is followed, and therefore provide information that is health- and safety-related and/or is connected to the functioning of society. Additionally, applicants need to assess alternative substances or techniques that reduce the overall risk to human health and the environment compared to using the respective SVHC. However, it is not known if the information provided in an application for authorisation (AfA) can be used in practice in an essential use assessment. Thus, the aims of this study are to (1) investigate if applicants for authorisation provide sufficient and relevant information that could be used to assess the essentiality of a use, and (2) identify potential gaps and challenges when applying the EUC to further suggest how this concept could fit in the REACH Authorisation procedure. Therefore, we currently analyse a pool of 140 AfAs based on pre-defined essential use criteria for data extraction. Our preliminary investigations suggest that applicants already provide relevant information for an essentiality assessment but that the scope of the overall assessment needs to be re-directed to justify a direct link between the specific function of a substance and its essentiality for health, safety and/or the functioning of society. Further, the hazards of alternatives are not sufficiently assessed to identify safer substitutes which might lead to regrettable substitutions.

##### **6.09.P-Mo393 Analysing Outcomes of the Current Regulatory Processes under REACH and the Stockholm Convention: Implications for Implementing the “Essential-Use” Concept**

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The “essential-use” concept was first introduced in 1987 in the Montreal Protocol to phase out the use of ozone-depleting chlorofluorocarbons, except for certain “essential” uses. In the Chemical Strategy for Sustainability, the European Commission proposed to use the concept in the decision-making processes of restriction and authorisation under REACH, with the aim to render the processes more efficient and aligned with societal needs. Much work is ongoing to develop criteria for implementing the concept in the EU chemical regulations. This study aims to inform the ongoing EU work on the criteria for the essential-use concept by providing an analysis of existing outcomes of restriction under REACH and of the listing under the Stockholm Convention. This analysis evaluates how existing regulatory outcomes differ from the “essential-use” concept and determines the reasons for eventual discrepancies. To that aim, this study analyses the rationale behind existing restriction outcomes under REACH and under the Stockholm Convention to determine the main reasons justifying granting a specific derogation. The REACH restriction dossier on intentionally added microplastics and the listing of PFOA under the Stockholm Convention served as additional case studies to check whether, by using the same information as evaluated in the respective scientific committees, an outsider would reach the same conclusion when applying the “essential-use” concept. The results suggest that in the majority of the cases derogations were granted for practical and technical reasons. It is unlikely that the implementation of the essential-use concept would influence these situations. However, in some cases, derogations were granted because it was believed that the costs of phasing-out the substance of concern for the particular use were greater than the benefits, or because the emissions, exposure and/or risks from the use of the substance of concern were judged negligible. In those cases, it is likely that the implementation of the essential-use concept would influence the decision-making and thus affect the conclusions regarding derogations.

##### **6.09.P-Mo394 Applying Quantitative Structure Use Relationships (QSURs) to Identify and Evaluate Functional Substitutes**

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Phillips et al. (2017) described Quantitative Structure Use Relationships (QSURs) as a tool that can “elucidate which features and properties of a chemical give rise to its function and identify chemicals with similar properties and features that could also fulfill that role.” Similar to how predictive models in toxicology assessments are used to screen potentially safer alternatives, QSURs



can help expedite risk management decisions on chemicals, particularly when data may be limited. When combined with exposure models, they provide a critical tool for rapid screening of potential alternatives and to compare chemicals that serve a particular function.

The Essential-Use Concept is a developing policy approach that, when implemented, aims to expedite the phase-out of “non-essential” uses of chemicals not necessary for the “health, safety, or critical functioning of society”. Here, QSURs may provide useful insights to efficiently support Essential-Use determinations by assessing whether safer functional substitutes exist and whether specific chemical functions or chemicals are indeed “essential”. In this presentation, we introduce QSURs and their applicability in supporting functional substitution, both when chemical options exist, as well as in the stage-gate process for new chemicals development. We also outline potential benefits of their use when combined with other screening level data such as New Approach Methodologies (NAMs).

#### **6.09.P-Mo395 Are Analysis of Alternative Methods Suitable for Poly- and Perfluoroalkyl Substances?**

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The upcoming European Poly- and Perfluoroalkyl Substances (PFAS) restriction is unprecedented in scope, thousands of substances will be covered. Many organisations and businesses are currently seeking alternatives to PFAS. When searching for alternatives to PFAS, hazard information about PFAS and about alternatives must be combined and a decision reached as to the most advantageous alternative. Are the current methods used to analyse chemical alternatives suitable for PFAS?

Over the past 40 years a number of Analysis of Alternative methods have been created. This includes decision-tree methods (e.g., Greenscreen, Cradle to Cradle), and weighted-sum methods (e.g., Multi Criteria Decision Analysis, Life Cycle Assessment, or Cost Benefit Analysis).

A decision-tree method and a weighted-sum method were investigated, with a particular interest in how these methods might be adapted to handle substances like PFAS. Hypothetical substance datasets and real substance datasets, from case studies in the scientific literature, were used to investigate the strengths and limitations of these methods.

The investigated methods showed considerable flexibility and there is also overlap between them. However, the ability to determine the acceptability of an alternative is not intrinsic to any method. Decision makers need to be aware of the decision rules and parameter settings that govern the application of these methods. Without appropriate awareness of decision rules to be employed as well as settings of method parameters, this flexibility can lead to unacceptable alternatives being erroneously categorised as acceptable. This poster concludes with recommendations as to the appropriate parameter settings to be used in these methods when they are applied to the analysis of chemical alternatives, and the analysis of chemical alternatives to PFAS in particular.

#### **6.09.P-Mo396 Implementing the Essential Use Concept For Intentionally Added Microplastics in the EU**

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Plastic pollution in the environment has been rapidly increasing for decades and the environmental impacts can be related to material size where macro-, micro (< 5mm) and nano (< 1mm) can have different fate, transport and biological interactions. Most microplastics originate from the degradation of plastic litter or release from normal use of polymer-containing materials (i.e., secondary microplastics), but microplastics are also intentionally added in a number of products (i.e., primary microplastics). While the reduction of secondary microplastics can be targeted through improved policies for mismanaged waste, other more targeted measures are needed that focus specifically on intentionally added microplastics. ECHA's proposed regulation of primary microplastics under REACH considers the wide variety of uses, physical and chemical properties, and applications collectively as a group. However, implementing the Essential Use Concept may help to balance environmental health, technical feasibility and innovation of new materials better than the currently proposed approach. Here we present a systematic framework of how microplastics could be categorized and regulated based on their use to stimulate innovation of more competitive and environmentally conscious materials. In some cases, substitutions are both politically and technically feasible. In other cases, substitutions may come with more uncertainty such as significant performance questions and monetary costs. A holistic and transdisciplinary alternatives assessment is therefore needed that considers microplastic risks and benefits in a particular use case as well as the existence, quality, costs, and hazards of replacement materials. This concept is illustrated by three case studies which reflect different tiers of essentiality; 1) microplastics use in rinse-off cosmetics, which score low on essentiality and have microplastic-free alternatives readily available, 2) microplastic infill material for artificial turf, which is debated in its level of essentiality and represents a complex substitution problem and 3) microplastic seed coatings, which are essential for agricultural productivity but still need to be replaced with more sustainable technologies. In all of these cases, the Essential Use Concept bridges the gap from the need to phase out risky materials to incentivizing informed decision-making towards sustainable and innovative alternatives.

#### **6.09.P-Mo397 Managing PMT/vPvM Substances in Consumer Products through the Concepts of Essential Use and Functional Substitution: A Case-Study for Cosmetics**

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Measures are needed to protect water sources from substances that are mobile, persistent and toxic (PMT) or very persistent and

very mobile (vPvM). PMT/vPvM substances are currently used in a diverse range of applications, including consumer products. The combination of the concepts of essential use and functional substitution has been proposed as a method to phase out substances of concern and support the transition to safer and more sustainable chemicals, a key goal of the European Commission's Chemicals Strategy for Sustainability. The aim of this work was to explore the potential of these concepts to support the phase-out of PMT/vPvM substances from consumer products by taking cosmetic products as an example. In this work, the number and type of cosmetic products containing PMT/vPvM substances were identified. Results show that circa 6.2% of cosmetic products available on the European market contain PMT/vPvM substances. The product group containing PMT/vPvM substances most often were hair care products. Based on their high occurrence, Allura Red (CAS 25956-17-6), Benzophenone-4 (CAS 4065-45-6) and Climbazole (CAS 38083-17-9) were selected as case-studies chemicals for the assessment of alternatives and essentiality.

The framework used in this study allowed to identify uses of Allura Red which are believed to be not necessary for the performance of some cosmetic products. In these cases, the use was considered as non-essential and the chemical should be removed from the application. For other applications of Allura Red, as well as all applications of Climbazole and Benzophenone-4, the technical function of the chemical was necessary for the product performance. However, safer alternatives were identified for all case-study chemicals, making their uses substitutable and therefore also non-essential. Further work is needed to refine the framework, e.g. by evaluating and comparing the reliability of experimental and QSAR hazard data, and by consulting different stakeholders to select the most relevant hazard endpoints and better deal with potential trade-offs of alternatives. This work shows that alternative assessment is a core step to phase out hazardous substances via the essential-use concept. It is therefore key that alternative assessment methods combine hazards-based considerations with life-cycle impacts to prevent problem shifting and assure that chemicals are used both in a safe and sustainable manner.

## 6.10 Regulation of Contaminants of Emerging Concern: Are We Missing Something?

### 6.10.T-01 Critical Review of Frameworks for Screening and Risk Management of Chemicals and Advanced Materials

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Regulatory programs and frameworks developed for the evaluation of safety and management of risks associated with chemicals and/or materials have evolved over the last half-century. Despite regulatory advancements, identification of new and emerging contaminants in the environment continues, suggesting a need for review of current approaches and strategies to better ensure safety of new chemicals and materials. To identify potential process improvements for facilitating early identification of potentially problematic substances and better inform risk management strategies, twelve existing frameworks relating to evaluation and management of chemical/material risk were reviewed. The selected frameworks represent a broad spectrum of regional, national, and international authorities and purposes, including pre-production evaluation of new substances, classification and hazard communication, identification of persistent pollutants, and identification of safer alternatives. Presentation will include an overview of commonalities and unique features for the reviewed frameworks and a summary of process improvement recommendations to enable more timely identification of potentially problematic substances. The conceptualized risk management framework based on these recommendations will also be presented.

### 6.10.T-02 Identifying Emerging Substances Gaps; A Prioritisation and Early Warning System for England

**Kerry Sims**, Helen Wilkinson, Nathalie Tonge, Mark Sinton, Jonathan RAY Newman, Ian Martin and David Brown, Environment Agency (England), United Kingdom

Reducing risks from a growing number of chemicals of emerging concern (CECs) is a recognised global challenge. The UK Government's 25-Year Environment Plan highlighted the need for early identification of CECs, so that effective intervention can be undertaken prior to damage being caused to the environment, wildlife, or human health. To address this chemical challenge, the Environment Agency (EA) have developed a Prioritisation and Early Warning System (PEWS) for England. The aim has been to establish a system which ensures appropriate regulatory focus on individual substances and groups of CECs. The PEWS process includes nomination and sifting of substances. Subsequently, substances are screened for potential risks to surface waters (freshwater and marine), groundwaters, soils, biota, and sediments and to human health via the environment. Screening is based on a suitable risk assessment, considering each substance in terms of readily available usage data, fate, toxicological parameters and environmental monitoring data. Screening CECs considers both exposure data (primarily based on EA monitoring data) and chemical hazard data. Screening is undertaken on batches of substances referred to as tranches. As of 2022, 215 individual substances have been screened in eight tranches. The limitations of a single substance approach to screening is recognised, so a group approach has also been adopted. Grouping approaches have been used on quaternary ammonium compounds, glycol ethers, linear alkylbenzene sulfonates, and retrospectively for science communication on neonicotinoids, pet flea and tick treatments, phenolic benzotriazoles, synthetic pyrethroids and flame retardants. The system concludes with chemicals being prioritised for environmental regulation where there is considered sufficient risk.

PEWS has demonstrated further monitoring requirements to clarify current environmental concentrations and increase certainty in prioritisation outputs. This presentation will describe the development and use of PEWS, illustrating some of the substances which have been screened for potential risks to date. It will also cover the links from PEWS to potential regulatory interventions where this process has been shown to be appropriate. PEWS has potential to be expanded to a UK-wide rather than England only system. There is opportunity to compare this approach to other decision-making frameworks utilised to prioritise regulation of CECs internationally.

#### **6.10.T-03 Considerations for Developing Risk Assessment Approaches to Manage CECs and Agriculture: PFAS And Livestock**

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Building resilience to climate change is a key driver for increasing use of recycled water and biosolids in agriculture settings. However, in 2019-2020 only 16% of wastewater in Victoria, Australia was recycled, with 84% discharged to oceans and waterways, in contrast approximately 87% of biosolids were land applied in agriculture settings.

Victoria has a significant peri-urban agriculture industry that provides a significant volume of the state's food produce and is expected to need to grow by 60% to meet population needs by 2050. In addition, Victoria may not have sufficient water supply to service its needs by 2028. Sourcing irrigation water from recycled water will have a significant impact on protecting key water needs.

The result is a need to balance domestic food production needs through access to recycled water and biosolids, with potential risks to the environment, human health and trade requirements. Emerging contaminants are challenging as their unique physico-chemical properties, toxicokinetic and toxicodynamic profiles and dynamic exposure pathways make development of proportionate regulatory frameworks difficult to establish. This is particularly the case when traditional approaches, that adopted aquatic or terrestrial ecosystem criteria, are not protective of agricultural objectives.

This presentation explores lessons learned from collaboration between research and regulatory organisations to support development of risk assessment and management frameworks for emerging contaminants in agricultural settings. Critical changes to standard approaches were necessary, enabling rapid adoption of research outputs to support regulatory actions. Key changes in approach included: 1) incorporation of detailed regional farming practice information to enable pragmatic advice; 2) development of dynamic livestock exposure models that capture physiological changes and seasonal factors that influence daily dose intake; 3) undertake serum measurement to identify and prioritize relevant PFAS; 4) early investment in species relevant toxicokinetic data (cattle, sheep) to support development of risk assessment and risk management frameworks; 5) early consideration of global trends for health-based guideline values to enable risk management frameworks that would be protective of future international trade needs. The learnings from this work are collated to reflect on unexpected findings and propose new approaches to addressing emerging contaminants in agricultural settings.

#### **6.10.T-04 Long-term Monitoring And Environmental Specimen Banking in Support of Environmental Policy**

**Jan Koschorreck<sup>1</sup>**, Annette Fliedner<sup>2</sup>, Bernd Göckener<sup>2</sup>, Alexander Badry<sup>1</sup>, Anna-Lena Kronsbein<sup>1</sup>, Kevin S. Jewell<sup>3</sup>, Robin Schütz<sup>4</sup> and Henrik Krehenwinkel<sup>5</sup>, (1)German Environment Agency, Germany, (2)Fraunhofer IME - Institute for Molecular Biology and Applied Ecology, Germany, (3)German Federal Institute of Hydrology, Germany, (4)University Duisburg-Essen, Germany, (5)University Trier, Germany

Environmental monitoring data play a key role in the Zero Pollution Ambition of the European Green Deal, setting the pace for more sustainable environmental policies and acting as a watchdog for their success. The study highlights the potential of environmental specimen banks (ESBs) to support environmental and chemical policy. The highly standardized samples from the ESB archives can be used to assess the state of the environment and monitor temporal trends in environmental pollutants and ecological conditions. Retrospective trend assessment using modern methods of environmental analysis allow to reassess the evolution of ecosystem health over the past decades, to better understand its current state in this context, and to derive more meaningful predictions for its future evolution. Recent ESB studies demonstrate the effectiveness of chemical management of legacy PFAS, polybrominated diphenylethers, chloroparaffins and plasticizers. Moreover, temporal trends reveal the rise of substitutes such as short-chain perfluoroalkyl substances, non-phthalate plasticizers, and long-chain chlorinated paraffins. Non-Target Screening proves to be a powerful method to complement target analysis and characterise the spatial and historical trends of chemical mixtures in the ESB samples and to identify chemicals of emerging concern that were previously unknown or overlooked, e.g. fluorinated pharmaceuticals. Similarly, new genetic methods are applied to ESB samples to fill knowledge gaps about biodiversity changes in recent decades: Data on shifts in species communities are now becoming available through eDNA metabarcoding and metagenomics, while population genomic analyses within species are applied to detect microevolutionary changes in genomes. The genetic data pave the way for comparisons with data for chemicals and other environmental stressors measured in the same samples. The German ESB samples are available to innovative research ideas in support of environmental management and policies.

#### **6.10.T-05 Towards a Zero Pollution Strategy for Contaminants of Emerging Concern in The Urban Water Cycle**

**Lian Lundy<sup>1</sup>**, Jan Hofman<sup>2</sup>, Leonard Oste<sup>3</sup>, Luis David<sup>4</sup>, Isabel Oller<sup>5</sup>, Andreas Matzinger<sup>6</sup> and Andrea Rubini<sup>7</sup>, (1)Lulea Technical University, Sweden, (2)University of Bath, United Kingdom, (3)Deltares, Netherlands, (4)Urban Water Unit, Laboratório Nacional de Engenharia Civil, Portugal, (5)CIEMAT, Spain, (6)Kompetenzzentrum Wasser, Germany, (7)Water Europe, Belgium

A central component of the European Green Deal is the EU Action Plan 'Towards Zero Pollution for Air, Water and Soil' which sets out an ambitious strategy to achieve a Europe where pollution is reduced to concentration levels that are not harmful to human health and ecosystems by 2050. From a water perspective, particular concerns relate to contaminants of emerging concern (CECs), defined here as chemical and biological substances that are not regulated under existing EU water quality regulations but that have been identified as having a potential negative impact on human health and/or environmental endpoints. The development of comprehensive measures to address CECs requires a detailed understanding of their sources, pathways and fate within water environments. As a contribution to achieving this aim and supporting delivery of the zero pollution action plan, the

Water Europe Working Group on Zero Pollution has drawn together international expertise from research and practice to co-produce a series of recommendations for evidence-based policy development and co-identify knowledge gaps that currently hinder achievement of a zero pollution water environment. Use of a source-pathway-receptor approach led to identification of eight key topics which between them encompass a range of environmental, technical and social aspects including point and diffuse sources and pathways, treatment technologies, analytical techniques and governance. Each topic sets out a concise overview of the state-of-art which is used to address a key question from policy support and research needs perspectives. As an example, in relation to principal pathways and their quantification, policy recommendations relate to the development of regulations for all discharge streams (including urban runoff, combined sewer overflows and unplanned discharges), and knowledge gaps associated with stormwater pollution concentrations and volumes and use of online sensors are identified. Through adoption of a cross-sectoral collaborative approach, this opinion piece provides an integrated research and industry perspective on opportunities to enhance regulation. It is further anticipated that implementation of these policy recommendations can significantly contribute to achieving the EU's zero pollution objectives and UN Sustainable Development Goals (e.g. SDG 6 water and SDG 11 Healthy cities), with knowledge gaps forming the basis of a cross-sectoral CEC-mitigation research agenda.

## **6.10.P Regulation of Contaminants of Emerging Concern: Are We Missing Something?**

### **6.10.P-Mo398 Post-Brexit UK Chemical Regulation and Policy - Does the Future Look Green?**

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On the 1st of January 2021, the United Kingdom (UK) formally exited the European Union (EU) and ceased to be subject to EU chemical regulation requirements. Prior to the UK's departure from the EU (i.e., Brexit), UK environmental policy and chemical regulation was largely controlled by a series of laws and commitments (i.e., regulation and directives) set out by the EU. The EU is often considered a leading regulatory state, however there is growing concern and scepticism amongst members of civil society, academia, and industry that current regulatory efforts are no longer fit for purpose. Brexit provides the UK government a unique opportunity to reflect on its regulatory policy, and reform its domestic legislation to ensure it is fit for the future. In its departure from the EU, the UK chose to abandon participation with EU regulatory regimes and institutions, instead opting for a narrative of deregulation and divergence. There are concerns that the UK is increasingly vulnerable to poor chemical regulation, and weak implementation, and enforcement. Not least due to the UK's historic reputation as the 'Dirty Man of Europe'. Through the review of relevant UK and EU documents related to the issue of chemical regulation and policy (published June 2016 - October 2022), this research attempts to understand the impact of Brexit on UK chemical regulation and policy. Evaluating whether the future of UK chemical regulation aligns with ambitions set by the EU, whilst highlighting areas where UK and EU chemical regulatory regimes have already begun to diverge. Standards and restrictions (in the UK) are already falling behind those of the EU. The comparative lack of regulatory capacity and resources to assess risk in the UK since its departure from the EU has resulted in the UK considering far fewer protective controls, hazardous substances, or restrictions than that of the EU. As the EU successfully develops and rolls out its own Chemical Strategy in line with the commitments made in its 'Green Deal for Europe' – the UK must decide if it wishes to align with the priorities and targets set by the EU or pave its own path. With divergence an inevitable consequence of two different legal regulatory regimes, the overwhelming concern is that the UK may become a dumping ground for chemicals that can no longer be produced or sold within the EU, whilst restricting trade and access to the European single market.

### **6.10.P-Mo399 Data Collection and Meta-Analysis on Plant Protection Products Occurrence in Soil at Worldwide Scale**

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The usage of chemicals including Plant Protection Products (PPPs) as crop yield booster has been recognized as the most efficient way for decades. According to food and agriculture organization (FAO), average use of pesticides on croplands has been increasing in the world since 1990. As a result, it is essential to monitor and investigate the occurrence of PPPs in different environment compartments. Although soil is generally under severe influence by PPPs applied on the croplands, monitoring the PPPs' residues has been underestimated in this compartment. With focus on currently used pesticides, in this study the situation of PPPs occurrence in agricultural soil in the world was investigated. The data of soil monitoring and surveys of 72 studies published for 50 years (1971 to 2020) from 34 countries in Europe, Asia, Americas, and Africa was collected and unified into one data set. Result of the meta-analysis on 376 individual active substances (281 parental compounds and 106 transformation products) involved in the prepared data set showed that organophosphate pesticides were the main matter of investigation in the majority of the studies in comparison to other chemical groups such as triazines, pyrethroids, and triazoles. The highest number of compounds (56 compounds) addressed in majority of studies (58 studies) were fitting in organophosphate chemical class. Organophosphates also had the highest overall maximum residue concentration (273.8 µg.kg<sup>-1</sup>). However, the overall detection frequency was higher for pyrethroids, equal to 37.4 %. Meta analysis at the active ingredient level revealed that mevinphos, diuron-desmonomethyl, esfenvalerate, and cyfluthrin were compounds with higher overall detection frequencies equal to 71, 69.9, 65.3, 62.7 % respectively. The results out of this meta-analysis are hoped to help clarification of the gaps in available data and actions needed toward regulations and official decisions on monitoring and screening PPPs in the soil around the world.

### **6.10.P-Mo400 What We Need to Know About Microplastics in the Environment**

*Joris T.K. Quik, Melvin Faber, Jeanine Ridder and Susanne Waaijers-van der Loop, National Institute for Public Health and the Environment (RIVM), Netherlands*

Microplastics are small plastic particles measuring less than 5 millimetres. They are found almost everywhere in the environment.

Laboratory experiments have shown that microplastics can have harmful effects on plants and animals, but it is not yet known whether these effects actually occur in the environment as well. This is partly due to wide variations in the shape, size and composition of the material. What is certain is that increasing amounts of microplastics end up in the environment. Solutions are needed to limit this and prevent harmful effects. More research into microplastics is crucial in this regard.

Many studies have already been done on microplastics, but there is no good overview of the findings. As a result, it is unclear what knowledge is lacking to come up with effective solutions. RIVM has therefore created an overview of the knowledge that is still needed in order to determine whether microplastics are harmful to the environment. This overview allows policymakers and other parties to make targeted choices regarding which research should be conducted first, for example to gain more insight into harmful effects. The results can then be used to better determine which measures are most effective.

RIVM spoke with university professors and experts for this study. They believe it is difficult to say which knowledge is needed first, because so much is still unknown. However, it is clear that the knowledge must be assessed in a cohesive manner in order to determine the harmful effects on the environment and develop effective solutions.

Information that is currently lacking for a proper environmental risk assessment includes how quickly microplastics break down in soil and water, and the differences per source in this regard. There is also insufficient data regarding the particle size and quantity at which microplastics are harmful. In addition to policy-based measures, attention must be paid to how behavioural change and innovation can reduce the amount of microplastics that end up in the environment.

In 2020, ZonMw drew up a knowledge agenda to gain a better understanding of the risks that microplastics pose to human health. Together with the present knowledge agenda, this highlights the areas where we need more knowledge about microplastics.

## **6.11 Risk Communication for Decision-Making: The Role of Community-Contact, Nature-Positive Outcomes, and Risk-Perception**

### **6.11.P-Tu417 Indigenous Practices That Influence Waste Management: Case Studies of Bushbuckridge Local Municipality**

*Siyabonga Madonsela, Cape Peninsula University of Technology, South Africa*

The African continent, just like other developing continents, such as Latin America and Asia, are struggling to manage solid waste. This transpires amidst municipal limited budgets, lack of proper equipment as well as the poor infrastructure especially within marginalized indigenous rural communities. It is for this reason that amongst other reasons local authority grapples with waste management in rural communities of developing countries. However, in addition to these challenges the problem with many local governments of developing continents if not all is to solely focus on indigenized conventional waste management strategies as the panacea of all waste management solutions. This in the process, relegates the waste management knowledge system of the indigenous communities into the background. To this end, the current study sought to investigate the indigenous practices that influence waste management within Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. Seven cases that would capture the spatial cultural diversity of indigenous communities across Bushbuckridge Local Municipality were selected for sampling. From the selected cases of indigenous communities, purposive non-probability sampling techniques were used to sample the groups of interest. Data was collected using the digital voice recording device. Data analysis was carried out using the thematic analysis approach. Whilst Microsoft Excel 2019 was used for data capturing and deriving simple descriptive statistics such as mean, statistically significant, and frequencies. Subsequently, charts, tables, and figures were derived and presented from Microsoft Excel 2019. Deductive and inductive logics were used in the interpretation of the study results. The results of the current study indicate that indigenous communities of Bushbuckridge Local municipality in the absence of formal waste management services from the local authority resort into Indigenous Knowledge system to manage solid waste stream. Taboos (71%), waste combustion (100%), animal feed (86%) and recycling (14%) are amongst some of the indigenous waste management practices espoused by BLM communities. The study argues that the sustainability accounting aspect of these BLM indigenous waste management practices is of paramount importance. Especially if these practices are to be merged into the sustainable integrated waste management strategies that informs the integrated development plans of the municipalities.

### **6.11.P-Tu418 From Microscopic Biodiversity and Micropollution workshop to Inclusive Citizen Science in the Basque Country**

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The Plentzia Marine Station of the University of the Basque Country presents a local citizen science and STEAM project entitled “**Attention! Don’t step on me!**” which pursues two main objectives: i) to get people close to the beach and increase their knowledge about the microorganisms inhabiting it; and ii) to raise awareness about the need of caring about marine environment. For this last, it is expected that the participants will experimentally address visible pollution, mainly macro and microplastics. Targeted audience ranges from youngsters students to any potential beach user, particularly pointing to groups not frequent in citizen science or public consultancy activities regarding environmental issues (i.e. functional diversity groups, women, and underrepresented cultural minorities). The plan is to sample water and sand at their closest beach for analyzing **microplastics, meiofauna and plankton** using scientific based methodologies. Although rocky shores host rich macroscopic fauna and flora assemblages, making them appealing sites for marine biodiversity dissemination, their complex topography and slippery substrates makes them risky and hampers access. On the contrary, sandy shores provide friendly environments for most potential

audience, yet, they have been poorly explored for dissemination as their biodiversity is mainly microscopic. Specific workshops will be directed to the leaders of the participating groups (i.e. outdoor activities staff, group coordinators, volunteers, educators) and the sampling will be adapted for collectives with special requirements. In this way, we maximize the extent of the project focusing on the person in charge of the guidance of the group.

The main result will be the activation, motivation and approach of the participants to do science and especially those people who live in unfavorable conditions or belong to minority groups. Moreover, the outcome information (data and images) obtained will be organized on a database that will contribute to expand the knowledge about species inhabiting the ecosystems under study, as well as, the abundance of microplastics. This information will be available following the Open Access policy so it could be used in the future for decision-making policies in the Basque Country.

#### **6.11.P-Tu419 The Future of Ocean Plastics: Designing Diverse Collaboration Frameworks**

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Experts and workshop participants discussed their experiences in the collaborative development and implementation of ocean plastic pollution projects held worldwide, guided by three main questions: (i) What is the role of scientists in a multi-stakeholder project?; (ii) How should scientists communicate with other stakeholders?; and (iii) Which stakeholders are missing in collaborative projects and why are they missing? This multidisciplinary, co-learning approach highlights the value of stakeholder engagement for ocean plastic projects with an end goal to identify and implement ocean plastic solutions via innovative technologies, informing policy,

community engagement, or a combination of all three approaches. The target outcomes of the workshop described in this paper include the identification of transdisciplinary (academic-stakeholder) engagement frameworks and specific suggestions that can serve as guidelines for the development of future plastic pollution projects.

#### **6.11.T-01 Holistic LCA of Impact and Benefit Assessment**

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Life Cycle Impact Assessment (LCIA) now only measures negative damages. Other sustainability methods that are gaining momentum are however also focused on bringing positive news about the sustainability benefits of products, services or buildings assessed. We have seen Cradle to Cradle, Blue Economy, and now Circular Economy and Nature Positive gaining momentum. Life Cycle Assessment (LCA) has half a century of experience in scientific calculation of sustainability impacts. It has been developed and standardized to a mature and scientific robust method that is been successfully used worldwide. Emerging positive sustainability assessment methods could benefit from the robust scientific background of LCA, and conversely LCA could incorporate assessing benefits into its methods.

Through illustration the authors compare various cohorts of benefit versus damage assessment methods. It then discusses case studies where both LCIA and Life Cycle Benefit Assessment (LCBA) results are used to assess the damages and benefits of project outcomes. The work demonstrates net damages as well as net benefits considering progress in development that is unsustainable, sustainable and regenerative.

The paper shows that design preferences could differ when benefits are included versus when they are excluded. It also shows how benefits can be communicated in a transparent way alongside of damages, without the risk of double counting or greenwashing.

#### **6.11.T-02 How to Manage and Confront with The Public Perception in Italian Sites in Contaminated by Per-and PolyFluoroalkyl Substances (PFAS)**

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An environmental scientist can not avoid to face up with the real world, investigating and finding problems and communicate them to all the stakeholders, both authorities and citizens. The role of scientist in risk assessment and communication is central and can have a weight on the risk perception of the stakeholders. A realist approach, which takes into account uncertainty in risk assessment, can be appreciated by authorities leading to minimisation of the problems (with less expenses as a consequence) and even in the worst cases to negationism. On the other hand, a precautionary approach could be forced to an alarmist and even a

catastrophist view. As a practical example I would like to present the history of the drinking water pollution by Miteni Company, in Veneto Region which was discovered in 2013 by our research group during a survey of the PFAS levels in the main river basins in Italy. Overall, the contamination extended over an area of 930 km<sup>2</sup> located inside the Brenta-Bacchiglione river basin and involving three different Provinces (Vicenza, Padova and Verona).

As main scientific actors in the problem at the beginning we chose to act as a scientific consultant for local and national governments, minimising public communication in order to not interfere with the official communication. Since 2018, we intensified contacts and exchanges with stakeholders, environmental association and citizens and we realised that official communication/information toward citizens was very poor and the relationship with authorities and their consultants was seen mainly in a logic of contrast and opposition. How to go beyond this logic? We would like to show some examples from real problems risen in the contaminated areas which regards *a)* the risk connected with the food consumption in the contaminated area; *b)* the derivation of Emission Limit Values for PFAS in industrial and wastewater discharges; *c)* the emissions from plant for thermal reactivation or incineration of GAC used in drinking water and in negative hydraulic barriers.

#### **6.11.T-03 Stakeholder Dialogue as Risk Management on a National Scale**

**Katharina Halbach, Janek Kubelt, Sue-Martina Starke, Ingo Warnke, Ines Flügel and Adolf Eisenträger, German Centre for Micropollutants, German Environment Agency, Germany**

A multi-year dialogue with stakeholders from industry, government, NGOs, water management and research has identified several tools to reduce the entry of micropollutants into water bodies in Germany. This process is currently further developed at the German Centre for Micropollutants which was established at the German Environment Agency (UBA). The main objective is to protect our inland water bodies and the raw water for drinking water production in cooperation with all concerned stakeholders. We will present two of our main tasks and evaluate their impact critically. 1. One main task is the assessment of the toxicological and ecotoxicological relevance of micropollutants. For the risk assessment, we apply the precautionary principle, i.e. we can classify micropollutants as “relevant” by concerning properties such as persistence, mobility or (eco)toxicity. We assess the micropollutants independent from the chemical’s registration. 2. Another main task is the engagement of communication between stakeholders affected by the relevant chemicals. The stakeholders are brought together in so-called “round tables”, where all measures to reduce environmental pollution are “put on the table” and discussed with focus on an extended producer responsibility.

Round tables discussing reducing measures for X-ray contrast agents, diclofenac and benzotriazole are finished. Several projects and awareness campaigns resulted from these. As an example, projects implementing the use of urinary collecting systems in Germany coupled with monitoring studies have started to reduce the inputs of X-ray contrast agents to water bodies.

Which criteria are useful to implement a round table for a micropollutant and which measures are realistically put on the table by the stakeholders? We will present the evaluation of the tool and how we are going to use it in the future.

We present a national effort to further reduce the entry of micropollutants in the water bodies and enhance the quality of drinking and surface waters. As it is, so far, a national process, it is important that information is available on the entry pathways in order to evaluate whether national efforts are promising. Our national process is a component between German, European legislation and future European strategies, e.g. Zero Pollution Action Plan, One Substance One Assessment. When being successful tools such as the round table may also be used at a European level.

#### **6.11.T-04 NaturePositive+ Ecolabelling & Transparency Reporting**

**David Baggs<sup>1</sup> and Delwyn Gloria Jones<sup>2</sup>, (1)CEO, Global GreenTag International, Australia, (2)The Evah Institute, Australia**

The needs to pull back from the brink of climate and biodiversity climate tipping points that society, the planet, and nature kingdoms face today are too terrible to fully countenance. The Global GreenTag International (GGTI) Certification Scheme under an ISO 19001 Certified Quality Management System has developed and launched a NaturePositive+ Declaration (NP<sup>+</sup>D) ecolabelling and transparency reporting program. The authors hope stakeholders find this work useful as manufacturers may apply each of the intrinsic NP<sup>+</sup>D assessments and metrics individually as a progressive rung on the ‘ladder of progress’ or achieving them together to move to and past net-zero carbon toward holistic Nature Positive outcomes.

GGTI has Certification Mark approvals in US, Canada, NZ and South Africa and via Australian Competition and Consumer Commission approval and registration in Australia. It is an externally verified ISO 14024 compliant 3rd Party Type 1 Ecolabel certification scheme, ISO 14025 Type III Environmental Product Declaration Program Operator and is an externally verified ISO 17065 compliant Conformance Assessment Body. GGTI programs are recognised by major rating tools in 172+ countries. The new NP<sup>+</sup>D product certifications and transparency declarations go beyond current thinking on circular economy, risk mitigation, ethical supply chain transparency, ingredient and product hazards and life cycle analysis (LCA). They report climate and biodiversity outcomes of restoring, regenerating, conserving and protecting ‘Natural and Technical Nutrient Cycles’ [10] and identify a pathway to nature positive product outcomes. GGTI has identified shortcomings in current approaches to these issues and developed NP<sup>+</sup>Ds as a solution for industry uptake. No other single certification yet provides the ease, scope, accuracy and depth of transparency also focused on changing real world outcomes towards Nature Positive.

The NP<sup>+</sup>D aims to provide markets with concepts and a solution to quantify both natural and technical cycles’ full scope and benefits as no certification has before as depicted in Figure 1. It adapts and integrates available metrics to provide one NP<sup>+</sup>D score for products, individual measures per issue with transparent reporting on: a. Human Health & Environmental Toxicity; b. Circular Resource Flows; c. Life cycle impacts and benefits; d. Ethical Supply Chains & Modern Slavery.

#### **6.11.P Risk Communication for Decision-Making: The Role of Community-Contact, Nature-Positive Outcomes, and Risk-Perception**

#### 6.11.P-Tu420 Communicating Evidence of Positive Outcomes

*Delwyn Gloria Jones<sup>1</sup> and Mathilde Vlieg<sup>2</sup>, (1)The Evah Institute, Australia, (2)Malaika LCT, Spain*

The UN Nature Positive Program provides a global impetus for regeneration. Scientific messages and media about accelerating anthropogenic climate change and biodiversity loss have had wide responses. Prevailing reactions, however include bad news, greenwash, disinformation and community indecision. Reports indicate the populace tuning out in confusion and inaction. Apparently, environmental science lacks the communication skills to support sufficient popular regenerative actions globally at grass-roots levels. If so, nature-positive policy and planning needs extended sightlines beyond zero damage to integrated benefits and net-gains.

The aim is to clarify qualitative concepts and challenges then review quantitative methods and case studies illustrating core scientific nature-positive numeracy and literacy to quantify security and viability. Life Cycle Benefit Assessment (LCBA) to model climate security, community wellness and supply viability gains is used alongside Life Cycle Impact Assessment (LCIA) modelling damage and loss

Results are shown of one biomass feedstock diverter case study's results cradle to grave. This commercial kitchen undersink grease diverter system separates and removes fat, oil and grease used to make biodiesel. Compared to no diverter there was one overall net gain and five benefits from reducing damages.

It is one example of how LCA can be more balanced by supplementing LCIA with LCBA to model security in climate, wellness, biodiversity and supply. The paper reviews other climate and biodiversity studies to illustrate net-positive recycled and renewable systems for products buildings, infrastructure and cities.

Whilst conventional environmental science and LCA has a negative bias it disempowers more effective communications and created barriers for nature-positive initiatives. LCBA offers community, government and business a new environmental science tool to measure gains in accelerating restoration and climate security.

Reaching to show gains well beyond zero loss enables a good news focus and truer market assessments. This reach offers hope to inspire wider public action knowing that only huge gains can restore planetary controls.

The authors recommend other environmental sciences extend the reach of allied concepts, tools and numeracy to yield positive messages. Capacity to report positive metrics can also reduce greenwashing prevalent bad news.

#### 6.11.P-Tu421 Environmental Communication: Different Channels Different Level of Pro-environmental Behaviours?

*Renata Dagiliute and Diana Miškelytė, Vytautas Magnus University, Lithuania*

The role of final consumers for reaching different environmental policy targets is crucial. Therefore, awareness rising and fostering pro-environmental behaviours is of importance. However, there is a variety of communication channels for environmental information, which can influence the activities undertaken. The study aims to analyse the influence of different environmental communication channels on pro-environmental behaviours indicated by EU citizens. Analysis is based on Eurobarometer survey conducted in 2020 and covering all EU member states of that time. Results reveal that television news remain the dominant source of environmental information (69.3%), followed by internet sources (36.7%) and newspapers (29.1%). Social networks are the source of information for some 18% EU citizens. Only 1.9% of respondents indicated museums, national or regional parks to be a source of environmental information. On the average respondents perform 4.2 of 14 analysed pro-environmental behaviours. Though scientific literature is the source of environmental information only for some 6.8% EU citizens, regression analysis indicate that books or scientific papers contribute to the number of actions performed most significantly. Second most significant source of information contributing to more pro-environmental action taken is internet sources (websites, blogs, forums), followed by newspapers and films and documentaries on television. Those who indicated books and scientific literature as a source of environmental information on average performed 5.9 activities, to compare to 4.8 activities of those receiving information from internet and 4.7 activities then information is received from the newspapers. Hence, preliminary results suggest that despite all sources might be of importance for environmental information provision, less employed ones should be promoted and used to raise awareness on environmental issues and corresponding behaviours.

#### 6.11.P-Tu422 Conveying Impact and Benefits of Recycled Sand

*Direshni Naiker<sup>1</sup>, Delwyn Gloria Jones<sup>2</sup> and Mathilde Vlieg<sup>3</sup>, (1)Gaia Conscious Consulting, South Africa, (2)The Evah Institute, Ecquate, Australia, (3)Malaika LCT, Spain*

This case study compares uncontrolled quarried river sand versus post-consumer recycled sand near Johannesburg. Results show net damages and benefits considering development in a biodiversity-rich nation. The aim is to show how environmental net-damage and net-benefit can be measured and communicated.

Uncontrolled quarrying gouges riverbanks, erodes soil, silts rivers, oil-pollutes water, disturbs aquatic habitat and downstream ecosystems and heavy vehicles crush vegetation. To produce re-usable fine sand uncontaminated building rubble comprising broken bricks and excavated soil is crushed on site. The functional unit is 60 years use building sand/m<sup>3</sup> filler cradle to grave for e.g. bricks, concrete or asphalt.

Methods include Life Cycle Impact Assessment (LCIA) of damage and loss and Life Cycle Benefit Assessment (LCBA) of benefit and gain. LCIA models loss of human health, ecosystems and resource accessibility due to pollution, climate change and land use change that deplete biodiversity, freshwater, minerals and fossil fuels. LCBA models security of human wellness, climate, ecosystems and resource supply, and land regeneration that repletes biodiversity, freshwater, mineral and fossil feedstock.

Table 1 shows biodiversity and climate damages and benefits of use of post-consumer recycled (PCR) sand compared to avoided quarried sand to 100-year horizons.



PCR product has benefits and losses. Its most significant biodiversity and climate security benefits outweigh other losses avoidable by reliance on renewable fuels. Other urban space, social and safety benefits arise.

A case study of damages and benefits of PCR sand use was demonstrated using the LCBA alongside LCIA. Comparing damage versus benefit can reveal net-losses and net-gains. The work demonstrates measurement and messaging essential to move from unsustainable to regenerative and sustainable development.

#### **6.11.P-Tu423 North Breton Island Reconstruction: An Example of a Successful Coastal Engineering and Natural Resource Restoration Project**

**Lawrence Malizzi**, *Ramboll*

North Breton Island is being restored under a contract to the U.S. Fish & Wildlife Service (USFWS) as part of the Deepwater Horizon Oil Spill Natural Resource Damage Assessment and Restoration (NRDAR) process. The project scope included permitting, investigation, construction design, planning, and construction implementation. The project is within the Breton National Wildlife Refuge (BNWR). The design restored beach, dune, and back-barrier marsh habitats within the island platform's footprint to support breeding birds, including brown pelicans, terns, skimmers, and gulls. The project team collected, compiled, analyzed, and managed topographic, bathymetric, sub-bottom, and magnetometer data of the island and the target borrow area and associated conveyance corridors, access channels, and project fill areas, as well as, sediment transport/velocity studies and impacts from recent hurricanes. The team also completed geotechnical, environmental, and cultural resource investigations in the target borrow area and within the project fill area and developing strategies to resolve potential conflicts with land rights, oyster leases, and oil and gas infrastructure. North Breton Island construction design included; approximately 76.2 acres (16,000 linear feet) of beach, 138.7 acres of dune, 137.3 acres of back barrier marsh habitat, a total island width of 1,100 feet, bounded by sloped foreshore and back barrier marsh platforms, an elevated dune platform of 8 to 10 ft. above sea level (optimum elevation to be determined through engineering and design) by 400 ft. wide at the base and 100 ft. wide at the top, a gulf-side beach 3 feet above sea level by 200 ft. wide, a landward back barrier marsh platform approximately 3 ft. above sea level and 500 ft. wide, and a targeted borrow area of approximately 677 acres. The construction of North Breton Island was completed in the beginning of 2022. Despite setbacks due to very active 2020/21 hurricane seasons. The General MacArthur dredge, owned by Callan Marine, pumped over 2.5 million CY of sand onto the Island. On a typical day, a crew of 40 to 50 people kept the dredge pumping around the clock and spread sand around the island using heavy earth moving equipment. In April 2021, Brown Pelicans began to nest in the Northern portion of the Island and are now nesting across the entire island. Communicating the success of the project is important to build consensus for similar projects in the future.

#### **6.11.P-Tu424 The Urgent Need for Nature-Positive Words, Ways and Weights**

**Rochelle Florence Bright**, *University of New England, Australia*

The ability of environmental scientists to engage, educate and inspire the general public will determine whether the world reaches the United Nations' goal of nature-positive by 2030. SETAC's advisory group on Science and Risk Communication (SCIRIC) alludes to an absence of public trust in environmental communications, and the media's focus on "disasters". They ask "Is it possible to tell positive stories and still show the relevance of our topics?"

This paper argues that it is difficult to tell positive stories when environmental measures and outcomes continue to be framed in negative language. Studies in communication across numerous fields highlight that while talk of disaster, or loss-framed messaging, might immediately get an audience's attention, it is positive communication of real benefits and gains that leads to action. Positive framing of potential and delivered benefits highlights what is possible, promotes autonomy, and fosters sustained engagement, all of which are fundamental to successful behaviour change.

A nature-positive goal therefore requires a nature-positive language, spoken by those who walk their talk. To claim their position as key climate change influencers, scientists, policy makers, and corporations must stop focusing on damages and improvement expressed as less worse outcomes, exemplified in Life Cycle Inventory Analysis (LCIA) vocabulary. Nature-positive language grounded in Life Cycle Benefit Analysis (LCBA) offers a way to translate scientific research, government policy, and corporate climate action into genuine, measurable gains for the planet and people.

#### **6.11.P-Tu425 Behaviour Test With Earthworms as an Additional Tool in Environmental Education About Soil Pollution Impacts For High School and University Students**

**Ondina Ribeiro**<sup>1</sup>, **Mateus Machanguana**<sup>2</sup>, **Cláudia Rubio**<sup>2</sup>, **Cláudia Ribeiro**<sup>3</sup> and **João Soares Carola**<sup>4</sup>, (1)UTAD, Portugal, (2)School of Life Sciences and Environment, University of Trás-os-Montes and Alto Douro (UTAD), Portugal, (3)Rua central de Gandra, Portugal, (4)CITAB, Portugal

Environmental pollution caused by human activities can affect the soil and its physical and chemical properties disturbing soil biodiversity and ecosystem functions. Thus, the issue of soil pollution must be integrated as part of environmental education in high school but also in university students using a hands-on approach to increase interaction with students and engagement.

Environmental education becomes important for students' reflection on environmental issues. Thus, this work aimed to involve students in environmental issues by giving basic theoretical concepts and practical component conducting a simple behavioural test with earthworms (*Eisenia fetida*) in lead-contaminated soil as an opportunity to real experience, improving kinesthetic learning. The students carry out the behaviour test, selecting 10 adult earthworms to place in a test container with two sections, one with a control soil and the other with a contaminated soil with different concentrations of lead (5, 50 and 500 mg/kg) with 3 replicates. After 48 hours, the students counted the number of worms in each section of container and the avoidance behaviour was determined. With this behavioural test, students were able to contact in a more direct way to soil pollution and its effects on terrestrial organisms, and also about lead concern for wildlife and Humans, namely kids and food webs. After 48 hours of exposure, an

avoidance behaviour of earthworms was observed for the highest concentration of lead (500 mg/kg). Almost all the organisms (9 in 10) were in the control soil with an avoidance percentage of 90%.

In conclusion, students learn theoretical concept related to soil pollution and hands on approach to assess lead effects. Students feedback show that the increase knowledge about soil conservation importance in food webs, ecosystem services, superficial water and groundwater quality. At the end they show better understanding about the direct and indirect impacts of soil pollution in wildlife and on human health. More work will be implemented to used questionnaires' to assess better the knowledge improvement before and after the task, including in future same task for younger students.

#### **6.11.P-Tu426 Pesticides and Their Metabolites in European Surface and Groundwater: Understanding Interacting Regulations and Approaches to Environmental Monitoring**

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Regulation designed to protect the environment and consumers is constantly evolving. This is driven by the need to consider new scientific knowledge as well as the desire for increased harmonization between different regulations. While the latter has the potential to simplify some aspects of regulatory requirements it also potentially leads to additional complexity, data requirements, and issues of interpretation.

One such area is environmental quality assessment utilizing environmental monitoring data. Interpretation of such data should be based on scientifically robust environmental quality standards to avoid incorrect conclusions and sub-optimal risk management decisions. However, defining substance specific quality standards or water treatment removal efficiencies requires additional effort as does ensuring they are applied in the correct situation, like only at drinking water abstraction locations which requires knowledge of where these are. This may lead to simplifying assumptions being made by regulatory agencies seeking to follow the guidance. While overly precautionary assessments based on simplification may be expedient and ultimately protective of the environment, they may have additional consequences like the withdrawal of economically important products with favorable risk-benefit profiles or the costs of continued monitoring for low risk substances.

This poster seeks to produce a synthesis of these interacting EU Directives assessing environmental monitoring data and the quality standards or regulatory thresholds they require to do so. In addition, the data required and guidance available to define these endpoints, the monitoring data and summaries to which they should be applied, and their intended use will be considered.

Gaps in available data and guidance to ensure consistent compliance assessment under these regulations will be highlighted. Recent developments like the proposed harmonized monitoring guidelines (SANTE 2020/12830) as well as revision of the Drinking Water Directive which, amongst other objectives, refocuses considerations of compliance to raw water alongside that of the tap of the consumer requiring the collection of data that identifies abstraction points as well as their source catchments will be factored in. It will also consider upcoming developments such as the proposed revision of the Water Framework, Groundwater and Environmental Quality Standards Directives.

#### **6.11.P-Tu427 Risk Management of Plant Protection Products: The Role of Farmers as Decision Makers on Site**

*Jonas Schartner<sup>1</sup>, Balthasar Smith<sup>1</sup>, Zvonimir Perić<sup>2</sup>, Jörn Strassmeyer<sup>2</sup> and Burkhard Golla<sup>2</sup>, (1)Federal Office of Consumer Protection and Food Safety, Germany, (2)Julius Kühn-Institute Federal Research Centre for Cultivated Plants (JKI), Germany*

Data-based decision-making processes are not only crucial in the context of risk assessment of plant protection products, but also offer enormous potential in the context of risk management. When deciding on the authorisation of a plant protection product, it must be ensured that the conditions imposed with the authorisation for the protection of the environment can be complied with a high degree of certainty and in a controllable manner. Improved data bases on local conditions that are available across the board can make the risk management mentioned much more efficient and target-oriented. At present, farmers have few options when it comes to implementing risk reduction measures. Ideally, the farmer on site should be enabled to make flexible and appropriate decisions on the use of risk reduction measures with the help of suitable support systems.

This poster presentation examines the common existing risk mitigation measures and the variables they contain that are based on factors in the field. Georeferenced data will be used to model the best possible representation of real field conditions. Emphasis will be placed on processing LiDAR data containing high resolution 3D information. The goal is to capture both the location and shape metrics of non-target areas of Plant Protection Products (PPP) applications (such as hedges, field margins and edges of embankment). In addition to the method, results for specific regions with data availability are presented. The derived information is provided at the end to best assist the farmer via online tools and web services. In a second part, the possibilities for further development are investigated, as it can be assumed that certain data will be available on national level in the near future. This concerns the protection of aquatic organisms, groundwater and the protection of insects or plants that are not the target of the crop protection measure. Various web services and tools, which are currently under development, will be examined and sample solutions already developed will be presented.

#### **6.11.P-Tu428 Toward a Greener Pharmacy: How to involve Doctors and Pharmacists in The Environmentally Conscious Prescription of Medicines**

*Francesca Cappelli<sup>1</sup>, Vitalia Murgia<sup>2</sup>, Stefano Polesello<sup>3</sup>, Grazia Ferrara<sup>4</sup>, Luisa Mattoli<sup>5</sup> and Massimo Mercati<sup>5</sup>, (1)University of Antwerp, Belgium, (2)Associazione Italiana Medici per l'ambiente ISDE Italia, Italy, (3)Water Research Institute - Italian National Research Council IRSA-CNR, Italy, (4)Apoteca Natura SpA, Italy, (5)Aboca SpA, Italy*

In recent decades, the combination of "pharmaceuticals" and "personal care products" (PPCPs) has been recognized as contaminants of emerging concern because their widespread and universal use leads to pseudo-persistence in aquatic

environments with ecological impacts on aquatic organisms, thus also posing a risk of selective pressure which can lead to a reduction in biodiversity, as well as the occurrence of antibiotic and viral resistance.

Doctors and pharmacists are the direct interlocutors of patients regarding the correct use of medicines and should give their contribution, each in their own field of action, in promoting the use of more environmentally friendly medicines. The present work presents an Italian pilot project for the information of doctors and pharmacists on the environmental risks of selected pharmaceuticals, through the production of a web-based collection of synthetic fact sheets on active pharmaceutical ingredients (APIs).

The first part of the project was devoted to the collection of occurrence data of APIs in the Italian water compartments, cross-referenced with sales data to generate a first list of about 80 APIs, for different therapeutical classes. For these prioritised compounds, the main physico-chemical and ecotoxicological data were collected to produce a synthetic Risk Score presented in substance-specific fact sheets which, in the phase of therapeutic prescription, can guide doctors and pharmacists in choosing more eco-sustainable medicines with equal therapeutic efficacy.

A similar approach could then be extended in a full-scale project to other compounds present in medicines such as excipients, personal care products and cosmetics, or to additives and colorants widely used in the food to raise awareness among both production and distribution technicians and consumers. The aim is to encourage proactive action to limit the use and when available, replace with products with a lower environmental impact to protect ecosystems and, consequently, human health.

#### **6.11.P-Tu429 Should What Is Joined Together by Chemistry Be Separated by Chemists? Studying Chloroalkanes in Particular and UVCB Substances in General**

**Chris Howick<sup>1</sup>, Andrew Jaques<sup>2</sup> and Richard Mariner<sup>3</sup>, (1)INOVYN Chlorvinyls, United Kingdom, (2)Chlorinated Paraffins Industry Association, (3)Euro Chlor, Belgium**

Since 2020 there have been significant regulatory developments for medium- and long-chain chloroalkanes (medium chain chlorinated paraffins and long chain chlorinated paraffins). Many of these developments are due to the different, global, approaches to understanding the environmental properties of this group of substances as they exhibit differences in their key variables, principally the length of the carbon skeleton and the level of chlorination. The challenge of describing environmental properties of substances such as these with complex compositions (some with over 100,000 components) is made apparent by the fact that both industry and regulators disagree on how to approach the challenge but, also, that different regulatory processes take different approaches as well. Such variations in approach to the categorisation of chloroalkanes are also seen in scientific literature, which has burgeoned in the past five years, but add to the confusion.

This work gives a summary on how substances with complex compositions have been evaluated by environmental testing on individual components as part of the EU REACH registration dossier and REACH substance evaluation programme. Such assessments show a general inverse relationship between the biodegradation rate and average chlorination level and yield results on specific commercial products. However some may use these same data to ascertain biodegradation properties of individual congeners and then use these to predict the properties of other products that may or may not contain such congeners.

We provide an overview on not only how the different approaches between industry and regulators results in different conclusions but also how distinct approaches from different regulators leads to diverse substance identities in different regulations. It also shows how a substance, shown to be inherently biodegradable in one test, can receive a persistent conclusion when assessed via a congener approach. The resulting regulatory decisions have practical consequences but also demonstrate that, in the coming years, product testing requirements are likely to accelerate.

#### **6.11.P-Tu430 Risk Should Not Be Made Complicated When the Hazards and Exposure Are Clear**

**Hans Peter Arp, Norwegian Geotechnical Institute (NGI), Norway**

Different stakeholders will approach scientific uncertainty differently. Scientists are often driven to reduce uncertainty; regulators are driven to act on uncertainty (e.g. by invoking the precautionary principle), and those that are responsible for causing the environmental stress often seek to use scientific uncertainty as basis for inaction or deniability. To cut through these different viewpoints, the tool developed by William of Ockham (1287-1347), referred to as Ockham's razor, can be useful for governance. Ockham's razor states that "Entities are not to be multiplied without necessity", meaning in practice for this context that if adding new variables/complexities does not lower the overall uncertainty or picture of a sustainability assessment, then they should not be added, as they will only make governance more complex, and less transparent. When it preventing harmful chemicals from entering the environment, this has an impact on the role of risk perception and communication. In many cases, the hazards and risk of exposure are clear enough, from the perspective of some stakeholders, to prevent a substance from allowing a harmful chemical to be produced for a certain use. However, those who can profit from such substances, either as a producer or a consumer, may be drawn into the discussion of uncertainty to keep questioning the risk models, as a strategy to keep allowing the harmful substances in the environment.

#### **6.11.P-Tu431 Is Discharge of Chemicals from Oil Extraction Offshore Harmful to the Environment?**

**Kirit Wadhia, Environmental, NOV, United Kingdom**

The discharge of produced water is of concern in view of potential environmental impact. Approach involving mere chemical analysis is no longer considered sufficient. holistic approach entailing whole effluent toxicity (WET) assessment from an ecotoxicological perspective has been increasingly advocated. Moreover, there is a need to employ a risk-based approach/assessment (RBA).

RBA has been instigated under OSPAR initiative and member countries have put in place appropriate implementation programmes. This presentation provides a comprehensive perspective of the UK RBA process. insight into the development of

the initial trial to the formulation of the current stepwise programme process involving chemical analysis, ecotoxicity testing, modelling and reporting.

Key considerations of the produced water risk management elements are discussed. The aspect of intrinsic toxicity contribution from production chemicals and naturally occurring substances is examined. Essential considerations of the key regulatory criteria and drivers are analysed.

The implication of the findings of the initial phase of the UK risk-based approach (RBA) programme are highlighted and how these impact future sustainable developments.

A definitive evaluation of the global RBA panorama is presented to rationalise top-down and bottom-up strategies conveying the global disparity that exists in the regulatory approach and existing infrastructure to consolidate the way forward.

The predictions are that major oil companies not only will continue with current level of production but will expand their operations that extract and refine fossil fuels.

The realisation is becoming all too apparent that in order to have a stable and safe planet reaching net zero emission is imperative. Whilst renewables are viewed favourable in terms of significant growth area, indubitably the issues and considerations pertaining to safe discharge of produced water remain at the forefront.

The intrinsic elements of importance for evaluation including production chemicals and naturally occurring substances play a key part. Methodology for assessment and inference from a global regulatory perspective lacks definitive approach and the disparity that exists inevitably compounds issues and instils lack of confidence.

Scope for pragmatic and prudent solutions to address pertinent issues will be highlighted.

#### **6.11.P-Tu432 Drinking Water Relevant Substances in the Meuse**

**Tineke Slootweg<sup>1</sup>**, **Gerdien van Genderen Genderen - de Kloe<sup>2</sup>**, **Bert Rousseau<sup>3</sup>**, **Thomas Oomen<sup>4</sup>** and **André Bannink<sup>4</sup>**, (1)*Het Waterlaboratorium, Netherlands*, (2)*Aqualab Zuid*, (3)*Water-link*, (4)*RIWA-Meuse*

The river Meuse is used as a drinking water source for 7 million people in Belgium and the Netherlands. RIWA-Meuse, the Association of river water works along the Meuse, aims for clean water in the river Meuse to guarantee the sustainable supply of impeccable drinking water. In 2007 RIWA-Meuse began to focus on specific substances which are relevant for the production of drinking water to advocate improvement of the water quality.

The goal of this study was to evaluate and update the current list of drinking water relevant substances (List 1) and identify candidate drinking water relevant substances from results of screening analyses and literature (List 2). The evaluation was performed based on measurement data from the monitoring stations and intake points along the Meuse in the period 2016-2020. Substances are considered as relevant if they fulfil a fixed set of criteria concerning i.e. their detection frequency, occurrence in concentrations above the target value set in the European River Memorandum, removal by water treatment, toxicity, odour/taste threshold and exceedance of the legal drinking water standards. To check if a substance fulfils the criteria monitoring data has to be available. Substances that are expected to be present in the Meuse, but are not monitored yet, are marked as candidate relevant substances and are recommended to be monitored by either a target analysis or screening.

This evaluation resulted in well-founded lists including 32 relevant substances and 33 candidate substances. The lists contain substances with a broad range of applications. Using the prioritization methodology substances that can be ranked according to their relevance for the drinking water production. All associated drinking water companies are committed to monitor the selected substances. The strong point of these joint lists is that it can be used for communication to stress the importance of the Meuse as a source for drinking water. Also it can be used to initiate targeted actions to reduce the emission of a substance in the Meuse.

#### **6.12.P SciComm Stars – The Science Awakens**

##### **6.12.P-Th404 Daphnia Detectives- The Case of the Pillowcase, Pegs and Pink Particles**

**Katie Reilly<sup>1</sup>**, **Hollie Marshall<sup>2</sup>** and **Iseult Lynch<sup>1</sup>**, (1)*University of Birmingham, United Kingdom*, (2)*University of Leicester, United Kingdom*

Science communication and public engagement are increasingly being recognised as an important element of research and the scientific process. Involving different audiences at various stages of the research process can increase their understanding of and engagement with the work undertaken, and enrich the overall research process. Through this, we can encourage behavioural changes which are beneficial for a range of topics, including environmental issues. Plastic pollution and microplastics (MP) have rapidly become a hot topic over the past few years, generating a lot of scientific and societal interest on the potential environmental and human health impacts of MP. Following from our research on the potential ways that MP can impact the environmental indicator species *Daphnia*, a suite of public engagement activities and events were undertaken. This has developed into the *Daphnia Detectives* project, which is a bank of open access online resources that cover the topic of MPs and *Daphnia*, from experimental design stages through to example results and data sets that can be used to explore data analysis themes.

Although the online content is designed to tie into the UK school curriculum as a way to bring real world and current examples of “research in action” into the classroom, it has the potential to be of interest to wider audiences. More recently, the project has also expanded into several in person events including science fairs and art installations.

A pillowcase, pegs and pink particles form an integral element of the *Daphnia detectives* activities, and are a good example of how everyday and specialist items can be blended to fit scicomm needs! ‘*Daphnia Detectives*- Science Festival edition’, is the focus of this case study, which follows the project from event proposal, assembly of a team of keen researchers, grant application for event consumables and resources, trial and error for the interactive table-top practicals, through to highlights from the event itself and key feedback from the public. This includes our personal highlights (table top DNA escape room and learning line) through to the challenges (“please can someone turn the power on for this part of the building...?”) and everything in-between!

## **6.12.P-Th405 Why Micropollutants in Drinking Water Have So Much to Do With Trust**

**Thomas-Benjamin Seiler**, Directorate, Hygiene-Institut des Ruhrgebiets, Germany

In 2021 I got invited by a water supplier to participate in a documentary on micropollutants in drinking water. They were looking for expert opinions on different aspects of this topic, from chemical analytical challenges over biological effects to options for treatment. Being no expert on drinking water in general, let alone any of these specific items, I offered them to talk about the science and in particular risk communication side - which they happily agreed to.

For preparation I received a list of questions I am going to be asked. I made my mind up about what I will say in response to these and used that opportunity to dig deep into 10 years of focusing on science and risk communication in environmental research. I found that what we have come up with in our work within the SETAC Interest Group on Science and Risk Communication (SCIRIC) since its foundation in 2015 is very helpful as a solid base to tackle challenges such as communication about micropollutants in drinking water.

I touched upon trust building, deficit models, confirmation bias and cognitive dissonance, ivory towers, emotional vs. fact-based opinion building and communication, uncertainty in science, risk vs. hazard and a lot more.

For me, as untrained but quite experienced science and risk communicator, this was a wonderful opportunity, the essence of which I would like to share with the wider environmental science community.

## **6.12.P-Th407 The Right Tool for the Job: Why and How to Adapt Your Science Communication for In-person and Virtual Events**

**Matteo Piumatti<sup>1</sup>** and **Francois Busquet<sup>2</sup>**, (1)Alertox Academy, Belgium, (2)Alertox, Belgium

More than ever, the challenges science communicators face nowadays remain daunting: they need to reach out to a variety of audiences with different interests, simplify the message without losing accuracy and engage beyond their community.

To overcome these hurdles, communicators need to be able to work with different tools since each one of them is best suited for specific situations. For example, a podcast might be very effective in describing the latest scientific discovery but does not allow the audience to interact directly and engage with the content. Similarly, depending on the communication objectives, in-person events could work better than virtual ones, even if they add a whole new layer of complexity.

Unfortunately, there is no secret recipe to master all the different actions within science communication. A great deal of trial and error is often required to learn which one is best for each situation and in which you feel more comfortable. Discussing with other science communication practitioners and sharing our stories is the best starting point to improve.

In this proposed talk, I will describe some of the formats encountered and practised since the beginning of my journey in science communication, as well as the recent experience with two innovative tools for science communication in toxicology: the live streaming show TOXstreams and the card game TATABox.

I will pay particular attention to the differences between in-person and virtual events, drawing from my direct experience. For example, my activities with the science outreach organisation pint of science Belgium and my participation in the “I love science festival” in Brussels during my professional work.

At the same time, I will describe my experience with podcasts and videos. More specifically, with my live streaming show TOXstreams, which merges elements from live talk shows, journalistic emissions and stand-up comedy.

As science communicators, we need to understand that each person we try to engage with is different, and we need to be comfortable with various tools to reach our audience. Sometimes it might not work perfectly, but only practising with new activities will help us and our community to grow.

## **Track 7: Moving Beyond – Cross Cutting Themes, Emerging and Transdisciplinary Topics**

### **7.01 Agriculture in the 21st Century: Balancing Food Security with Environmental and Public Health**

#### **7.01.P-We424 Regulatory Drivers and Testing Standardisation**

**Kirit Wadhia**, Environmental, NOV Inc, Stromness, United Kingdom

Impacting different sectors, regulations constitute key aspect of policy making. It is imperative for adequate protection of the environment to address key criteria underpinning the fundamental objectives. Formulating the core policy and monitoring mechanism, evaluation protocols employed for assessment and inference play a vital role.

Standardisation Organisations (ISO, OECD, ASTM) are at the forefront of practices adopted worldwide, advancing the frontiers of innovative methodologies utilised for assessment, inference and policing.

Use of standards significantly contribute in ensuring services (and products) fulfil criteria of reliability, safety and quality. This allows for implementation of robust and fit-for-purpose standards and are instrumental in facilitating international trade and developments. Certifications are vital for business as strategic tools to increase productivity and enhance efficiency. They allow for effective implementation of statutory measures.

The work of organisations such as ISO and OECD assist in establishing international norms. These Bodies contribute to the identification of prominent issues and the formulation of evidence-based solutions facing environmental and economic challenges of varying magnitude. Priority issues on the global agenda are tackled with international co-operation by member countries.

International organisations play an important role as standard setting bodies in supporting regulatory co-operation.

The conveyance is aimed to provide insight into the function and modus operandi of global organisations at the forefront in developing, maintaining and innovating international standards. Furthermore, exemplifying the application of standardised protocols implemented in different sectors and in regulatory frameworks.

#### 7.01.P-We425 Biostimulants: a complex ecotoxicological challenge

*Nadine S Taylor<sup>1</sup>, Callum Burgess<sup>1</sup>, Peter Godfrey<sup>1</sup> and Sarah Clarke<sup>2</sup>, (1)Cambridge Environmental Assessments (CEA), United Kingdom, (2)RSK ADAS, United Kingdom*

There is evidence that biostimulant products can benefit arable and field grown horticultural crops, however, there are large knowledge gaps regarding the environmental safety and ecotoxicology of these products. Few studies look at the potential ecotoxicological effects of biostimulants, and those that do tend to focus on the individual component(s) of these products. Whilst acute exposure data, to individual biostimulant components, are generally above the Environmental Hazard Classification threshold of 1 mg/L, there is evidence of sub-lethal, long-term chronic effects. There are instances of toxicity at high and sustained application levels, which is of concern when there are no product usage regulations in place. Several biostimulant categories have exhibited indirect toxicity effects, e.g., increased bioavailability of metals and other bound toxins. Many biostimulants also have pesticidal properties, useful with regards to the dual action of plant growth promotion and crop protection, yet a concern that the non-selectivity of any pesticidal effects could be detrimental to non-target species. Similarly, the use of products that contain growth promoting bacteria will arguably influence the existing natural community composition, structure and function.

Here we discuss the challenges involved in assessing the risk of biostimulant products to the environment. Where environmental risk assessments for products, such as plant protection products (PPPs), can base their decision-making process on a single active ingredient, biostimulant products are made up of various components, in highly variable compositions, with additional interactive effects, that all need to be considered to accurately assess any adverse impact they may have on the environment.

Standard risk assessment approaches, on a product-by-product basis, would provide a clear measure of the ecotoxicological impacts of a given biostimulant product, and if they are greater or less than the given application rates or predicted exposure concentrations following application. However, indirect toxicity, such as additive or synergistic effects of other contaminants present in the system, and the use of other products that are likely to be applied alongside biostimulants in real-world scenarios, also need consideration.

#### 7.01.P-We426 Overview of Recent Biological Pesticide Evaluations in the EU: Fate and Ecotoxicological Data

*Claudia Vaj<sup>1</sup>, Anne Alix<sup>2</sup>, Sian Ellis<sup>2</sup>, Elizabeth Collison<sup>3</sup> and Sara Lamperti<sup>1</sup>, (1)Corteva Agriscience, Cremona, Italy, (2)Corteva Agriscience, Abingdon, United Kingdom, (3)Corteva Agriscience, Cambridge, Cambridgeshire, United Kingdom*

The aim of this poster is to provide a summary of biological pesticides which have been evaluated at EU level since the introduction of the data requirements according to Regulation No. 283/2013. The review will assess the key findings of the EFSA conclusions to highlight data gaps and areas of uncertainty to identify potential recurring trends related to the environmental risk assessment of biological pesticides. The review will include note of any specific approaches such as exposure based waivers or novel study design which have been successfully accepted. The presentation will also relate the identified data gaps to i) the current status of the testing methods specific for biologicals, and ii) the new Commission Regulations (EU) 2022/1438 – 1441 (applicable for microorganisms from November 2022), to determine where further work is required to appropriately address these gaps. We hope this work stimulates continued discussion with regards to the development of scientifically relevant testing strategies and risk assessment schemes for biological plant protection products, facilitating the availability of alternative solutions for farmers in the EU market.

#### 7.01.T-01 A random forest machine learning model for prediction of NMs root uptake and translocation in plants based: application of nanoinformatics in sustainable and precision agriculture

*Georgia Melagraki<sup>1</sup>, Dimitra-Danai Varsou<sup>2</sup>, Panagiotis D. Kolokathis<sup>3</sup>, Iseult Lynch<sup>4</sup> and Antreas Afantitis<sup>5</sup>, (1)Entelos Institute, Larnaca, Cyprus, (2)NovaMechanics MIKE, Greece, (3)Department of ChemoInformatics, NovaMechanics MIKE, Greece, (4)University of Birmingham, United Kingdom, (5)NovaMechanics Ltd., Cyprus*

Engineered nanomaterials (NMs) have unique physicochemical features whose potential is being explored in precision and sustainable agriculture for instance, as nano-fertilizers to improve crop yield and nutritional quality, as nano-pesticides which can target pests more effectively with fewer pesticides, minimizing effects on soil health and biodiversity while increasing soil function and nutrient cycling by boosting soil microbiota, or to increase plant resilience to extreme conditions by giving plants improved functionalities that help them cope with environmental stress from climate change. Computational modelling or nanoinformatics can provide critical insights for optimisation of agricultural productivity. Key to all nanoinformatics approaches is the need for a deep understanding of the fundamental mechanisms of interactions of the NMs with the plant-soil and plant-animal systems, and how these interactions change as a function of the NMs properties, the environmental conditions (e.g., soil properties, climate and weather conditions) and the nature of the crops or livestock systems under consideration. The combination of experimental data on soil conditions, plant species, climate conditions, and NMs physicochemical properties, and application of Machine Learning approaches, may be able to predict both the impacts of NMs on the agricultural system (on plants and soil) and the effects of the agricultural system to the NMs (transformations, distribution, and bioavailability). This will contribute to the development of nano-agrochemicals that combine both optimized safety and enhanced functionalities. Here, we present a machine learning model to predict the uptake and transport fate of NMs in soil and hydroponic systems utilising a panel of metal and metal oxide-cored NMs: Ag, CeO<sub>2</sub>, CuO, Se, Si, TiO<sub>2</sub>, ZnO, exploring the drivers of plant uptake expressed as the root concentration factor (RCF) and translocation factor (TF). The literature dataset was enriched with a computational descriptors calculated using only knowledge of the NMs structure and composition. A Random Forest machine learning model was developed using Enalos+ functionalities via the Isalos Platform, and its accuracy in external validation ( $R^2=0.81$ ) was the reliability of predictions ensured

through a clear definition of the domain of applicability. Application of this model will support the development of NMs for agricultural applications that are safer and more sustainable by design.

#### **7.01.T-02 Ensuring Sustainability of Nanotechnology in Agriculture through Practices of Responsible Innovation**

**Khara Grieger**, *North Carolina State University*

Researchers, industry, regulators, and other stakeholders are interested in harnessing the potential of nanotechnology and engineered nanomaterials to develop more sustainable food and agriculture systems. For instance, nano-fertilizers and nano-pesticides may offer more efficient delivery of agrochemicals while reducing environmental run-off, and nano-encapsulated veterinary medicines may provide more animal friendly vaccine delivery for livestock. At the same time, we know from previous experiences that food and agriculture sectors are known to be among the most sensitive to public scrutiny, particularly related to the use of new and novel technologies, and require consumer acceptance to be deemed truly sustainable. For these reasons, various organizations have developed initiatives to support the responsible development of nanotechnology, including applications in agriculture systems, with most research efforts related to understanding potential environmental, health, and safety (EHS) risks along with efforts to evaluate ethical, legal, and societal implications (ELSI).

Our research integrates findings from EHS and ELSI studies on nanotechnology in food and agriculture (nano-agrifoods) and taps into a growing body of literature focused on responsible innovation of new technologies. Broadly, responsible innovation is a governance approach to innovating new technologies in a way that incorporates social and ethical concerns, stakeholder involvement, and issues of trust and transparency along with practices of inclusion, responsiveness, anticipation, and reflexivity. Through funding provided by USDA/NIFA, our research identifies i) barriers to conducting responsible innovation in nano-agrifood sectors, and ii) actions to address stakeholder concerns related to nano-agrifoods. Outcomes from this work are then coupled with insights from the broader literature on responsible innovation to iii) identify best practices for responsible nano-agrifood innovation that are also relevant for other emerging food and agriculture technologies. This presentation reports on key results related to these research areas and also discusses future directions to support sustainability of nanotechnology in agriculture sectors. Overall, these findings may be relevant for researchers, scientists, industry, and policy-makers to help pursue responsible innovation and ensure sustainability of emerging food and agriculture technologies more generally.

#### **7.01.T-03 Nanobiotechnology-based Strategies for Enhanced Crop Stress Resilience**

**Jason C. White<sup>1</sup>**, **Lijuan Zhao<sup>2</sup>**, **Jorge Gardea-Torresdey<sup>3</sup>** and **Arturo Keller<sup>4</sup>**, (1)Connecticut Agricultural Experiment Station, (2)Nanjing University, China, (3)Chemistry and Biochemistry, University of Texas El Paso, El Paso, TX, (4)University of California, Santa Barbara

Low use and delivery efficiency of conventional agrichemicals is a significant impediment to maintaining global food security, particularly given that a 60-70% increase in food production is needed by 2050 to support the projected population. Further confounding these efforts is a changing climate, which may force increased cultivation of crops under more marginal and stress-inducing conditions. Thus, novel and sustainable strategies for enhancing food production are needed all along the “farm-to-fork” continuum. Nanobiotechnology approaches to engineer crops with enhanced stress tolerance may be a safe and sustainable strategy to increase crop yield. Under stress conditions, cellular redox homeostasis is disturbed, resulting in the over-accumulation of reactive oxygen species (ROS) that damage biomolecules (lipids, proteins, and DNA) and inhibition of crop growth and yield. However, delivering ROS-scavenging nanomaterials (NMs) at the appropriate time and place can alleviate abiotic stress. Importantly, ROS-production in living cells carries both costs and benefits. When present below a threshold level, ROS can mediate redox signaling and defense pathways that foster plant acclimatization against stress. We find that many NMs are ROS-triggering, such as nanoscale Cu, Fe, S, and CuS, but these materials have the potential to be judiciously applied to crop species to stimulate defense systems, prime stress responses, and subsequently increase the biotic and abiotic stress resistance of crops. This knowledge can be used to engineer climate-resilient crops. It is also clear that the ability to effectively tune nanoscale material structure and composition will be critical to maximizing positive impacts, including significantly reduced amounts of agrichemical use while simultaneously enhancing yield.

#### **7.01.T-04 What are the Roadblocks for the Commercialization of Nanoagrochemicals today?**

**Fabienne Schwab**, *University of Fribourg, Adolphe Merkle Institute, Switzerland*

Nanoagrochemicals are an emerging solution to solve environmental issues due to poorly targeted agrochemical application and their toxicity to non-target organisms. As with every emerging technology, nanoagrochemicals have pros and cons. Here, an overview will be given on the opportunities and limitations of nanoagrochemicals based on the most recent research on the efficiency, mode of action, and delivery of active ingredients. In addition, an analysis of nanoagrochemical definitions and their regulatory significance, and the challenge to compare them with conventional products will be presented. Finally, based on these findings, the commercialization potential of different nanoagrochemicals will be discussed, including their hazard potential and scale-up cost. Overall, the most significant challenges for nanoagrochemicals today are to optimize them for field application, produce them at a competitive cost, and overcome the regulatory obstacles to register products containing nanomaterials.

#### **7.01.P Agriculture in the 21st Century: Balancing Food Security with Environmental and Public Health**

##### **7.01.P-We427 Nematicidal Effect of Ultrasound-Extracted Cyanotoxin**

**Emily Yi Wai Chiang**, *School of Engineering, University of Guelph, Canada*

Root-knot nematodes are one of the plant-damaging plant-parasitic nematodes in agriculture reducing crop yield food quality. The conventional solutions to control these plant-parasitic nematodes involve inherent negative impact on the environment as well as

inefficacy after year after year of continued use, so biological control is among the most promising sustainable alternatives. The present study examines the nematocidal potential of two microalgal species: *Trichormus variabilis* and *Nostoc punctiforme* against a root knot nematode *Meloidogyne hapla*. *T. variabilis* is known to produce a neurotoxin, anatoxin-a, and *N. punctiforme* is known to produce a hepatotoxin, microcystin. The main objective of this study is to investigate the effect of ultrasonication on the extraction of secondary metabolites from these two microalgae, and the secondary objective of this study was to check for their soil nematode suppressing potential. Ultrasound results in cell wall disruption of the microalgal species, thus resulting in enhanced release of secondary metabolites. Microalgal biomass was treated with an ultrasound probe at 50 % amplitude, 20 kHz frequency, using water as the extraction medium, for 5–30 min. The extraction efficiency was determined in terms of the total chlorophyll content of the extract. Microscopic images of the treated cells were also investigated to gain insight into the effect of the ultrasonication time on the cell morphology. The nematized control soil contained 80 nematodes per kg soil, and upon addition of *T. variabilis* extracts (TV), 100 % nematodes are reduced. With *N. punctiforme* extract (NP) addition, 66 % nematode inhibition is observed. For a combination of the two microalgal extracts (TV + NP), 91 % nematode inhibition is observed. Paired t-test indicated that the number of nematodes in microalgal extract treated soil are significantly different ( $p < 0.05$ ), thus implying that the microalgal treatment could result in nematode suppression. The higher inhibition for TV treatment can be attributed to the concentration of the cyanotoxin present in the extract. Also, the predominant toxin produced by TV is anatoxin-a and by NP is microcystin, hence the ratio of these metabolites in the extract dominates the nematode suppressing strength.

#### **7.01.P-We428 Effect of Plant Biopesticide-spiked Soils on Earthworms and Soil Enzymatic Activity, With Biochar as an Ameliorant**

Hein Smith<sup>1</sup>, Oladayo Amed Idris<sup>1</sup>, Owen Rhode<sup>2</sup> and Mark Maboeta<sup>1</sup>, (1)North-West University, South Africa, (2)Agricultural Research Council-Grain Crops, Spain

Plant biopesticides are not only effective at managing agricultural pests, but they are also culturally acceptable and do not significantly damage the ecological system or exacerbate environmental pollution. *Bidens pilosa* L. and *Tagetes minuta* L. are examples of plant biopesticides used for crop protection. This study aimed to investigate the potential effects of the biopesticides on earthworms and soil enzymatic activity with reference to the conventional pesticide, fenamiphos, and the efficacy of biochar in the amelioration of plant biopesticide-spiked soil. A sublethal endpoint was considered using *Eisenia andrei* as a bioindicator. The avoidance behaviour, relative growth rate (RGR), and reproduction success were evaluated following OECD standard guidelines. Genotoxicity was evaluated using a comet assay and soil enzymatic activity spectrophotometric methods. The RGR and fecundity of *E. andrei* in fenamiphos-treated soil were significantly lower than in soil treated with plant biopesticides (*B. pilosa* and *T. minuta*). Surprisingly, treatments with biochar had a lower earthworm RGR compared to treatments without biochar. However, biochar does not have a significant impact on the reproduction of *E. andrei*. On the contrary, biochar supplemented treatments showed no DNA damage, suggesting biochar prevents genotoxicity. There were no significant differences in the alkaline phosphatase and urease activities in all treatments, including soils supplemented with biochar. But  $\beta$ -glucosidase activity increases in soil treated with 25 and 100 mg/kg Fenamiphos and 250 mg/kg of *T. minuta*. According to the findings of this study, fenamiphos has a greater negative impact on soil organisms than plant biopesticides. Further, biochar is not an efficient ameliorant for the studied plant biopesticides and pesticide.

#### **7.01.P-We429 Challenges in the Registration of Natural Substance Pesticides From An Ecotox Point Of View**

Jutta Müther<sup>1</sup> and Aleksandra Zakryś Zalewska<sup>2</sup>, (1)Ecotoxicology, GAB Consulting, Germany, (2)GAB Consulting, Germany

Under the Farm to Fork and Biodiversity strategy, the use and related risk of chemical pesticides as well as the use of the more hazardous pesticides should be reduced by 50% by 2030. Pesticides consisting of natural substances, like plant extracts, fatty acids, and oils are one tool to achieve this ambitious goal.

The lack of adequate testing procedures and of updated evaluation guidance (as recently published for Microbials) leads to risk assessors' conclusions which put natural substances like fatty acids, plant extracts or oils at a disadvantage.

This abstract highlights challenges related to the ecotoxicological risk assessment, common to most natural substances, which were faced meeting the data requirements of Reg (EC) No 283/2013 and No 284/2013. Recommendations for improvements to facilitate a reasoned evaluation and registration of natural compounds are presented.

The data requirements for Reg (EC) No 1107/2009 are targeted primarily at discrete, single-compound, chemical pesticides. The properties of natural substances are significantly more complex and difficult to fit into this schema. The evaluations for the broad variety of natural compounds are commonly handled on a case-by-case basis. Our experience is that this leaves both regulators and notifiers uncertain of how to provide and evaluate the requirements specified in EU regulations.

From the ecotoxicological point of view the most important challenges are: 1) The physical-chemical properties of natural substances and the resulting need to adapt existing testing methods. 2) The performance of accompanying analytical work for dose verification. 3) Interpretation of achieved endpoints and risk assessment calculations.

In this context, different aspects like lead component approach for multi-component plant extracts, biological degradation of active ingredients in test systems or extremely high application rates for fatty acids and oils are addressed.

Repeated testing, with modified testing methods trying to overcome the above-mentioned difficulties lead to inhomogeneous data packages. The interpretation and evaluation of this data is not possible in a consistent, straightforward way. In most cases it is not possible to prove safe use of a natural substance pesticide by standard risk assessment calculations and a weight of evidence approach needs to be applied. For both regulators and notifiers, the question remains how much uncertainty and deviation from the usual evaluation process is acceptable.



#### **7.01.P-We430 Practical Challenges of Data Generation to Address Regulatory Requirements for Microbial Organisms**

**Anne Alix<sup>1</sup>, Sian Ellis<sup>1</sup>, Claudia Vaj<sup>2</sup> and Sara Lamperti<sup>2</sup>, (1)Corteva Agriscience, United Kingdom, (2)Corteva Agriscience, Italy**

The data requirements present a range of practical challenges when generating data for microorganism biopesticides. Without available standardized test guidelines specifically for microbials and due to the varied nature of their activity, assessment will often need to be made using specifically tailored studies. Toxicity and effects endpoints may therefore differ between studies and study design should be adapted to the nature of the mode of action and to establishing conditions in which the micro-organisms may produce toxic effect. For agrochemicals the assessment relies on established standardized guidelines and methodologies, which are not sufficient to cover all the data needed for micro-organisms and so a more adaptable review process is required. To ensure an appropriate evaluation of the exposure and toxicity, there needs to be a balance between addressing deviations from the data requirements while ensuring the studies are necessary and fit for purpose to cover the action and behaviour of the microbial. Weight of evidence assessments using different sources of information may prove to be more appropriate than studies alone. The focus of this poster presentation is to identify some issues for consideration when developing appropriate testing strategies for microorganisms in their use as biological pesticides.

#### **7.01.P-We431 Regulatory Challenges in the EU to Providing Biological Solutions to Deliver the Green Deal**

**Martin Tilbrook, Andy Chadwick and Clare Lane, ERM Regulatory Services, United Kingdom**

Despite the European Commission's/Parliament's stated aim of replacing conventional chemical pesticides with biological pesticides, there are a number of regulatory barriers to overcome in registering biological pesticides in the EU. ERM has recently been involved with preparing a number of biological pesticides for submission to EU regulatory authorities, including botanical, biochemical and microbial products.

Current guidance under Regulation 1107/2009 requires that microbial pesticides are also additionally subject to 'Part A' data requirements (i.e. as applied to conventional chemical pesticides) due to their production of secondary metabolites that occur naturally in the environment.

European Commission guidance on botanical active substances states:

*Estimated exposures of the (components of the) botanical active substance should be compared to the natural exposure situations in different relevant environmental compartments (water, soil, air). The risk can be considered acceptable when estimated exposures are lower or similar to the natural exposure situations and no unacceptable effects occur on relevant non-target organisms.*

However, as well as natural background level comparisons, regulatory authorities have required comprehensive exposure assessments for soil, groundwater and surface water designed for conventional chemical pesticides for botanical pesticides.

The exposure assessments showed an acceptable risk to groundwater and to soil and surface water-dwelling organisms. ERM has received positive feedback on the approach from the authority responsible for the evaluation of the first of these biopesticides as it progresses through the EU's regulatory system.

Due to potentially high application rates required for some biological pesticides for efficacy, groundwater modelling can predict levels above the EU hazard cut-off of 0.1 µg/L. Since this cut-off is not based on risk, it is considered that it should not apply for natural substances with existing background levels of exposure.

Due to the above obstacles, cost and extended timeframes, registration of biological pesticides lags behind the US, despite the objectives of the SUR/Green Deal, echoed by the EU Parliament recently during a seminar on the Sustainable Use Regulation (13 October 2022).

#### **7.01.P-We432 Long-term Exposure of Bumblebees (*Bombus terrestris*) in Semi-Field Studies**

**Markus Persigehl<sup>1</sup>, Mareike Beinert<sup>1</sup>, Florian Ballhaus<sup>1</sup>, Guido Sterk<sup>2</sup>, Paraskevi Kolokytha<sup>2</sup> and Janna Hanegraaf<sup>2</sup>, (1)Tier3 Solutions, Germany, (2)IPM Impact, Belgium**

Biological plant protection products (Biopesticides) play an increasingly important role for sustainable crop production following the farm-to-fork strategy set in 2020 in the EU. Biologicals (bacteria, fungi, viruses etc.), like all other active substances used in plant protection products (PPP) have to fulfil the approval criteria laid down in (EC)1107/2009 concerning the placing of PPP on the market. However, due to their different modes of action, test designs have to be adapted in order to better serve the profile of the biological.

One essential adaptation in study designs is the duration of the exposure period. In pollinator semi-field effect studies (e.g. OECD75/Eppo170 for honey bees or icppr ring-tested study designs for mason and bumblebees) the exposure period in the tunnel is set to 7-10 days. This is in most cases not sufficient for testing biologicals as they continue growing and thus exposure increases with time.

For testing biologicals on the large earth bumble bee (*Bombus terrestris*) we developed a semi-field design with an exposure period of up to 30 days. Additionally, modified R&D hives were used to ensure a maximum synchronized development of bumble bee colonies. Further adaptations of the hives reduce disturbance during colony assessments. Details of the test design will be presented.

#### **7.01.P-We433 Toxicity Data of a New Generation Biopesticide Based on the Activity of a Peptide Regulating The Expression of a Digestive Enzyme in Insects**

**Libânia Queirós<sup>1</sup>, Matilde Moreira dos Santos<sup>2</sup>, Artur Sarmiento<sup>2</sup>, Sandra Afonso<sup>2</sup>, Mari Gigauro<sup>2</sup>, Mykola Rasko<sup>2</sup>, Camila Campello<sup>2</sup>, Rui Ribeiro<sup>2</sup>, José Paulo Sousa<sup>2</sup>, Estevão Mano<sup>3</sup>, João Transmontano<sup>3</sup> and Tiago Luz<sup>4</sup>, (1)CWT - TURISTRADER, Portugal, (2)University of Coimbra, Centre for Functional Ecology, Portugal, (3)Neovictus, Campus Politecnico, (4)Department**

Some peptides have the potential to regulate the expression of essential enzymes for the digestion process in insects, which may constitute a mode of action to control insect expansion. Biopesticides based on this mode of action may represent a paradigm change in pest management as they are biodegradable and might have high specificity for the target organism, not affecting other species that are beneficial for crop systems. Based on this principle, the BIOSTARV project foresees the search and identification of peptides with the potential to inhibit the expression of trypsin in the intestine of target agricultural pests in southern Europe. A decapeptide produced in the females' ovarian follicle of the *Aedes aegypti* mosquito blocks the trypsin biosynthesis, an enzyme essential for the digestion in the *A. aegypti* larva, and has been used as biocide in several Asian Countries (AC) in a commercial formulation. The application of this decapeptide (TMOF- trypsin modulating oostatic factor) in the larvae leads to the inhibition of their growth, preventing the larvae from reaching the adult state by starvation. The same decapeptide had a similar effect in the larvae of insects of the genera *Anopheles* and *Culex*, which suggests that TMOF has the potential to successfully control insect pests in crop areas. Aiming to evaluate the potential of a biopesticide based on the activity of a decapeptide similar to TMOF to be used in agricultural scenarios, the toxicity of TMOF and the biocide commercialized in AC was evaluated for non-target organisms following the list of assays included in the data requirements of the European Union for the registration of active ingredients and Plant Protection Products. Detailed results of laboratory acute and chronic ecotoxicological tests with aquatic organisms (e.g. *Daphnia magna*, *Chironomus riparius*, *Pseudokirchneriella subcapitata*), honey bees (adult acute - oral and contact - and chronic oral exposure and larval repeated exposure tests), non-target arthropods other than bees (*Aphidius rhopalosiphii* and *Typhlodromus pyri*) and soil organisms (*Eisenia andrei*, *Folsomia candida* and *Hypoaspis aculeifer*) will be presented. Overall, lethal and chronic toxicity data for aquatic organisms have shown that test substances are more toxic to *C. riparius* than to *R. subcapitata* and *D. magna*, while for honey bees, toxicity has been found only in adults chronic exposure and larvae toxicity tests.

#### 7.01.P-We434 The Newly Implemented Regulations for Microbial Active Substances and Products – An Ecotoxicological Perspective

Cozmina Link-Vrabie, Anne Steenbergh, Emily McVey, Mascha Rubach, **Devdutt Pratap Kulkarni**, Ingrid Bremmer, Renske van Eekelen and Jacoba Wassenberg, Board for the Authorisation of Plant Protection Products and Biocides, Netherlands

The regulatory framework for plant protection product (PPP) containing micro-organisms is set by the Plant Protection Product Regulation (PPPR; Regulation (EC) No 1107/2009). This regulation states that a PPP can only be authorized when the active substance has been approved, the product is sufficiently effective, and use of the product does not have harmful effects on human health and have no unacceptable effects on the environment. These conditions should be met for all PPPs independent of the type of active substance (microbial or chemical). The data requirements for the active substance and the PPP are set in separately implemented regulations. The approval criteria for the active substance are given in an Annex to the PPPR itself (i.e., Annex II to Regulation (EC) No 1107/2009). The decision and evaluation criteria are provided in an implementing Regulation commonly referred to as the 'Uniform Principles' (see Article 29, paragraph 6 of the PPPR).

In August 2022, newly implemented regulations, which provide updated requirements and criteria for microbial PPP, were adopted. The requirements and criteria were updated to reflect the development of scientific insight and experience gained from the evaluation of PPP containing micro-organisms. These amended regulations with the relevant requirements and criteria for microbial PPP already apply from the 21<sup>st</sup> of November 2022.

This poster aims to provide further explanations on these newly implemented regulations, focusing on effects on non-target organisms. Based on our experience as a Regulatory Authority, we will provide information relevant for the different stages of dossier preparation and the assessment of effects on non-target organisms.

#### 7.01.P-We435 Systemic Acquire Resistance (SAR) Inducers as an Alternative to Conventional Pesticides – Environmental Hazard Assessment

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Systemic acquired resistance (SAR) is a natural long-lasting defence mechanism of plants. SAR can be induced by natural signalling molecules (e.g. salicylic or jasmonic acid) or by synthetic analogues e.g. benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester (BTH). Such induction of natural defence mechanisms can be elicited in broad variety of crops and acts against a wide range of pathogens (bacteria, fungi, viruses). The induction of resistance is also associated with the stimulation of plant metabolism that provides long-lasting beneficial effects like increased yield. Unlike classical pesticides, SAR inducers do not act directly on pathogens but rather stimulate the plant to defend itself. SAR inducers are therefore an alternative to plant protection agents, which might reduce the use of pesticides or even substitute them. BTH - a known SAR inducer - is poorly water soluble and therefore difficult to apply on the fields by spraying. It is however, much more soluble in water in the form of ionic derivatives.

We prepared eleven ionic BTH-derivatives (BTHCOO<sup>-</sup>) and tested their environmental impacts: ready biodegradability, cytotoxicity and aquatic toxicity (against luminescent bacterium *Aliivibrio fischeri* and water flea *Daphnia magna*). None of the tested compounds proved to be readily biodegradable which is largely due to the fact that BTH itself is not biodegradable, yet five derivatives showed appreciable levels of degradation. The tested SAR inducers were characterized by a wide range of IC<sub>50</sub>/EC<sub>50</sub> values in cytotoxicity and (eco)toxicity indicating a highly diverse toxicity. However, a carboxylic acid derivative coupled with a cholinium cation showed a better environmental hazard profile than the parent compound due to lower toxicity and higher, but probably only partial, biodegradability. This compound was also shown to be one of the most effective SAR inducers in tobacco

plants infected with Tobacco Mosaic Virus where the protective actions against virus lasted for over a month after application of this novel SAR inducer.

The development of new SAR-inducers offers a possibility to actively include environmental impacts as one of design criteria using so called 'benign by design' approach. Some BTH-derivatives offer an improvement both in terms of functionality and environmental hazard, while others are significantly more hazardous. The type of counterion accompanying the BTHCOO<sup>-</sup> played a pivotal role in defining environmental hazard.

#### **7.01.P-We436 Impact of Copper Oxide Nanoparticles Combined With Indole-3-acetic Acid in *Pisum sativum* Plants and Seeds**

**Loren Ochoa<sup>1</sup>** and **Jorge Gardea-Torresdey<sup>2</sup>**, (1)Environmental Science and Engineering, University of Texas at El Paso, (2)Chemistry and Biochemistry, University of Texas El Paso Nanotechnology in agriculture has evolved and helped in seeking solutions to increase food production at a global scale. Copper oxide NPs (nCuO) a potential nano-pesticide in the agricultural sector. Its interaction with phytohormones such as indole-3-acetic acid (IAA) in plants remains to be completely understood. This study aims to evaluate the interaction of nCuO and IAA in green pea (*Pisum sativum*) plants within both plant and the nutritional components in its fruit. Green pea plants were grown to full maturity in soil amended with nCuO, bulk CuO (bCuO), and cupric chloride (CuCl<sub>2</sub>) at 50 and 100 mg/kg of soil and IAA at 0, 10 and 100 µM. Results from the whole plant indicated that treatments with 10 and 100 µM of IAA in conjunction with all the copper treatments reduced the growth of plants by 23% and 34% accordingly. IAA at 100 µM with Cu treatments changed the nutrient accumulation in tissues. The green pea pods compared to the control showed an increase in the content of Fe (258%) and Ni (325%) in treatments of nCuO at 50 mg/kg. Treatment of bCuO 100 mg/kg increased Ni content by 275%. Cu content increased in stems by 84% and 78% in treatments with IAA at 10 µM with nCuO at 100 mg/kg and bCuO 50 mg/kg, respectively. A reduction of Ca of 32% and 37% was shown in treatments with IAA at 100 µM with nCuO and IAA at 100 µM with bCuO, respectively. The nutritional evaluation of the green pea seeds showed that IAA at both concentrations increments the sugar content by 20%. While IAA at 10 µM does increased protein content by 33%. Fe content in seeds was observed to increase by 41% in 50mg/kg of bCuO treatment, and by 42% in 100 mg/kg nCuO treatments both in the presence of 100 µM of IAA. A reduction of B by 80% was shown in treatments with IAA at 100 µM with nCuO at 50 mg/kg. The combination of IAA and Cu-based compounds can impact the production of green pea plants by reducing the plant number and biomass, though the nutritional quality and production is not affected. To further expand the knowledge of what is already been discovered in the interaction of exogenous phytohormone IAA and nCuO in pea plants, a comparison with the previously mentioned findings will be conducted with a chitosan-based fertilizer intended as supplement for nCuO. This slow-release fertilizer will be evaluated in conjunction with different amounts of IAA to evaluate if the rate of release affects the aforementioned variables within the whole plant and fruit components.

#### **7.01.P-We437 Tunable Release of dsRNA Molecules into Plants from Sustainable Nanocarriers**

**Washington da Silva<sup>1</sup>**, **Raja Muthuramalingam<sup>2</sup>**, **Karol Barroso<sup>3</sup>** and **Juliana Milagres<sup>4</sup>**, (1)Virology, The Connecticut Agricultural Experiment Station, (2)Plant Pathology, The Connecticut Agricultural Experiment Station, (3)Plant Pathology, CAES, (4)CAES

Virus resistance has been induced in plants by the application of exogenous double-stranded RNA (dsRNA). However, the effectiveness of applying naked dsRNA on leaves is short-lived - dsRNA is quickly assimilated by plant defense mechanisms and is also degraded by environmental factors. We are investigating the potential of using nanoparticles to sustain an effective and prolonged delivery of dsRNA into plants with the long-term goal of developing innovative technologies to control plant virus infections. We performed small RNA profiling analysis in tobacco plants infected with potato virus Y (PVY) and synthesized dsRNAs from PVY genomic regions with a high abundance of virus-derived small interfering RNAs. The application of the synthesized dsRNAs resulted in 100% protection against PVY on tobacco plants for up to five days. Currently, we are synthesizing and characterizing different nanocarriers to protect dsRNAs from degradation to prolong the "vaccine effect" of those molecules on plants for pathogen protection.

#### **7.02 Better Safe than Sorry: Safe, Sustainable and Circular Chemicals, Materials and Products along their Lifecycle**

**7.02.T-01 The Role of Life Cycle Assessment in the Safe and Sustainable by Design: Lessons Learnt From a Case Study**  
**Davide Tosches<sup>1</sup>**, **Carla Caldeira<sup>1</sup>**, **Debora Fino<sup>2</sup>** and **Serenella Sala<sup>1</sup>**, (1)European Commission Joint Research Centre, Italy, (2)Department of Applied Science and Technology, Politecnico di Torino, Italy

The action plan of the Chemicals Strategy for Sustainability foresees the development of criteria for safe and sustainable by design of chemicals and materials, which integrates aspects from the domain of safety, circularity and functionality of the chemicals or materials considering them throughout the entire life cycle. To support the development of such criteria a framework was developed integrating different dimensions of the sustainability: safety, environmental, social and economic. In particular, environmental sustainability is addressed using Life Cycle Assessment (LCA).

However, the ambition of the SSbD poses some relevant challenges to the LCA community in order to operationalize the assessment towards supporting the sustainability transition of the chemical sector. These challenges are explored in a case study. This study aims to present and show possible ways to address the identified key challenges.

The case study refers to the application of the LCA part of the SSbD framework to a set of non-phthalate plasticisers used in food contact material selected considering the most common ones allowed for food contact according to European regulation. A phthalate plasticiser (DEHP) is also included to be used as a possible reference material. The plasticisers are considered for the use in a specific packaging application: the manufacturing of PVC based gaskets for metal lids.

The LCA was performed applying the Product Environmental Footprint (PEF) method. The scope of the study is a cradle to grave analysis of the plasticisers, which function is to provide the proper softness to manufacture a polyvinylchloride gasket. The modelling of the life cycle is performed using literature data complemented by the use of a process design software and primary data from companies.

During the development of the case study several challenges were so far identified, such as: the need to define application specific criteria, the availability of primary data in the chemical industry.

The identification of challenges in the use of LCA supporting the SSbD framework is crucial to develop the latter into a decision-making tool. This case study was pivotal to illustrate that challenges in the use of LCA are related to all steps, from goal and scope to life cycle impact assessment. Overarching issues are related to data availability and quality, and the definition of a proper application-specific benchmark as a basis to set criteria and requirements.

#### **7.02.T-02 Safe and More Sustainable Alternatives in the Innovation Funnel; A Case Study on Biobased Materials in Cosmetics**

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The Safe and Sustainable-by-design (SSbD) concept can be used to guide the early design phase in the innovation stage-gate process of chemicals and materials to minimize hazardous effects on human health and the environment along the life cycle while considering a specific function. Here we aim at identifying a screening methodology for the SSbD-steps from existing and newly developed tools to be applied early in the innovation stage-gate process. To define the methodology and explore suitable tools we use a case study on nanocellulose in cosmetics, a biobased material with high dermal exposure for consumers. This adds complexity to the assessment like identifying and managing trade-offs relevant to the transition to a bio-economy.

Nanocellulose is a renewable material derived from the most abundant biopolymer in nature and can have a wide variety of potential applications due to its interesting properties. The choice of starting material, pre-treatment, and process affect safety and environmental impacts from raw material extraction, production, use phase, and end-of-life. A cross-disciplinary team of professionals from chemistry, formulation chemistry, toxicology, life cycle assessment, and techno-economy has been formed to explore the four assessment steps in SSbD-criteria. Questions that will be addressed are for example: when and what (bio)material and chemicals should be prioritized for analysis, to what extent is a sustainability analysis required early in the design phase, how can *in silico* predictions be used for complex biomolecules and what *in vitro* assays should be prioritized for chosen endpoints and risk assessments. To answer these questions a combination of *in silico*, *in vitro*, literature and experimental data will be used. For the screening methodology, we suggest both new and existing tools from several disciplines including alternative assessment.

#### **7.02.T-03 Chemicals in Textiles: Can We Ensure a Safe Circular Economy in the Textile Sector?**

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The textile industry has grown extensively in the past decades and is now one of the largest industries in the world, generating more than 92 million tons of waste every year. This tremendous production goes along with severe environmental impacts, including greenhouse gases emissions, use of freshwater supply and arable land, and generation of severe chemical pollution. Thousands of tons of pesticides and fertilizers are used every year, additionally to a myriad of chemicals used throughout the production chain, such as lubricants, solvents, bleaches, dyes, processing aides, and water and stain repellents. The textile industry is currently unsustainable, and systemic changes must be made. One lever to tackle this issue is the transition to a circular textile economy, which must ensure users' safety and the absence of hazardous substances in recycled textiles. Concern has been raised on the (in)ability of current regulations to provide sufficient protection regarding recirculating materials. The aim of this presentation is to discuss potential regulatory loopholes and suggest solutions to ensure better safety of circular textiles. First, we present the toxicity profile of 711 substances that may be found in textiles. For selected examples, we also present how chemicals have been regulated over time. Based on this analysis, we identify regulatory gaps that could lead to hazardous chemical exposure, then present potential solutions to ensure a safe transition to a circular textile economy. Among the studied chemicals, we identified carcinogenic-mutagenic-reprotoxic (CMR) substances, endocrine disruptors, skin and/or respiratory sensitizers/allergens, and chemicals completely or partially lacking hazard data. Based on the case studies of PFOS and dicofol, we identify issues for safe textile recirculation and show they stem from the fact that regulations are based on chemical risk assessments that do not consider chemicals' specific hazardous properties and/or are not adapted for circularity. To address these issues, we argue that an improved risk assessment of chemicals should consider both chemicals hazardous properties (*e.g.* endocrine disruption, carcinogenicity) and product's multiple life cycles, instead of being limited to the product's end-of-life stage. In this perspective, we also discuss the recent ecodesign Regulation, how it could improve the circularity of the textile sector, and which key point should be included in the future delegated acts.

#### **7.02.T-04 Application of Life Cycle Assessment (LCA) Methodology to Drive Environmental Sustainability Improvements in the Manufacture and Delivery of Pharmaceuticals**

*Andy Whiting and Alexander Mullen, Astrazeneca UK, United Kingdom*

Across the product value chain, there is increased stakeholder interest for holistic information on the environmental impact of medicines, and the regulatory environment is shifting towards zero pollution, low carbon sustainable products. To make strategic decisions about how to best reduce the environmental impact of products, many pharmaceutical companies are making increased use of life cycle assessment (LCA).

LCA is an internationally recognised method of quantifying environmental impacts across the entire value chain of a product (ISO 14040 and 14044). It identifies hotspots within a product's life cycle where the impacts are greatest. Thus these areas can be prioritised and targeted, and potential solutions can be tested through scenario analysis.

However, there remain challenges to overcome with the LCA approach. The development and manufacture of a new medicine is a complex endeavour and it can be difficult to assess sustainability in early development when data availability is limited. There is a lack of standardisation within the pharmaceutical sector, leading to LCA results not being shared more widely due to fear of unfair comparison. Common rules, tools and scoring systems need to be developed for the sector. Additionally, despite considering many environmental impacts, there are issues associated with the complexity and uncertainty of LCA's ecotoxicity impact category and its relationship with existing environmental risk assessment approaches.

## **7.02.T-05 Assessing the Growth of The Field of Alternatives Assessment Over The Last 10 Years**

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Alternatives Assessment is a tool for the informed substitution of identified chemical hazards in consumer products and industries, with the intent of avoiding "regrettable substitution". The field of Alternatives Assessment has grown steadily over the last few decades and became particularly established with the publishing of the 2013 Royal Society of Chemistry's "Chemical Alternatives Assessments" book and the subsequent publishing of the 2014 U.S. National Research Council's "Framework to Guide the Selection of Chemical Alternatives". Since then, Alternatives Assessment methodologies, frameworks, tools, case studies, and a community of practice have developed. In 2018, Tickner et al. published the article "Advancing Alternatives Assessment for Safer Chemical Substitution: A Research and Practice Agenda" (<https://doi.org/10.1002/ieam.4094>), in which they amassed input from 40 experts to outline specific needs for the field in five critical areas: hazard assessment, comparative exposure assessment, lifecycle considerations, decision-making, and professional practice. Here, we assessed how the Alternatives Assessment field has grown in these five areas since 2013.

Alternatives Assessment literature covering frameworks, methods, tools, policy analyses, reviews, and case studies from 2013 through 2022 were collected for review using specific search terms in PubMed and GoogleScholar. Results were categorized by critical area and sub-area, literature type, and literature form. Over 200 sources were identified, including ~100 peer-reviewed journal articles. Identified sources also included government reports, white papers, non-peer-reviewed commentaries, books, theses, conference papers, and seminars. Together, these sources provide insights on advancements in the field for each of the five critical areas, as well as other aspects of the alternatives assessment process that are important and still evolving. This presentation will highlight these advancements, as well as the needs that still remain to be addressed in the field going forward.

## **7.02.P Better Safe than Sorry: Safe, Sustainable and Circular Chemicals, Materials and Products along their Lifecycle**

### **7.02.P-Mo401 Safe And Sustainable by Design Chemicals and Materials: A Framework For Criteria Definition**

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The EU Chemicals Strategy for Sustainability (CSS) aims at contributing to the safeguard of human health and the environment as part of an ambitious approach to move towards a zero-pollution and toxic-free environment. A key action defined in the CSS is the development of criteria for safe and sustainable by design (SSbD) chemicals. SSbD aims at facilitating the industrial transition towards a safe, zero pollution, climate-neutral, and resource-efficient production and consumption, addressing adverse effects on humans, ecosystems and biodiversity from a lifecycle perspective. To fulfil these ambitions, there is the need to develop a framework for the definition of criteria for SSbD chemicals and materials to steer innovation towards the green industrial transition, foster substitution or minimisation of the production and use of substances of concern, and minimize impact on human health, climate, and the environment. This study presents a SSbD framework developed in line with these objectives to support the design and development of safe and sustainable chemicals and materials within the research and innovation phase.

The framework is composed of two components: a (re)design phase in which design guiding principles and indicators are proposed to support the design of chemicals and materials, and a safety and sustainability assessment comprising 5 steps that can be carried out sequentially or in parallel, depending on the specific purpose of the exercise. The steps include: hazard assessment of the chemical/material (Step 1), human health and safety aspects in the chemical/material production and processing phase (Step 2), human health and environmental aspects in the final application phase (Step 3), environmental sustainability assessment along the entire chemical/ material life cycle (Step 4), and social and economic sustainability assessment, providing (Step 5). The framework will be tested with case studies to identify and collect bottlenecks, and measures to address them will be explored. Insights gained from the testing phase will support the refinement of the framework and criteria.

### **7.02.P-Mo402 Challenges and Opportunities for Safe and Sustainable by Design Assessment of Chemicals and Materials**

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The importance of chemical products for modern society is undeniable since they deliver essential services throughout many sectors. However, along their life cycle, chemicals are responsible for water, air, and soil pollution and the related impacts, e.g. on climate change, biodiversity loss, and human health. Hence, there is a clear need for a new approach that guarantees the safe and

sustainable design, production, use, and waste management of chemicals. In this direction, the European Commission published the Chemicals Strategy for Sustainability (CSS) aiming to address those issues. Within the CSS action plan, the Safe and Sustainable by Design (SSbD) concept is introduced as a principle for the substitution and development of new chemicals, materials and products. Safety assessment and environmental, social, and economic sustainability assessments should be combined with design principles to evaluate whether a chemical is safe and sustainable, already in an early stage of its development.

Several studies already identified challenges for SSbD assessment, for example, data availability and quality, methods for data scale-up, modelling aspects of chemical functionality. To move towards an operational framework, those challenges need to be addressed by the scientific community, supporting implementation into business and policy. The goal of this study is to support the development of an SSbD assessment framework by answering the following research question: *What are the most important challenges and opportunities to create an operational SSbD assessment procedure for chemicals that can be implemented by the chemical industry?* For each step of the SSbD assessment framework, we identified potential challenges, both scientific and operational, that need to be further addressed and we provided recommendation toward the operationalization of the SSbD framework.

Among all the challenges, the introduction of a “Step 0” is crucial as an entry point to further guide the SSbD assessment. To do so, the development of archetypes with specific guidelines could contribute on providing support the operationalization of the SSbD framework. Further recommendations on other challenges provided through this study might guide further improvement toward the operationalization of the SSbD framework.

#### **7.02.P-Mo403 Interdisciplinarity as the Backbone for Sustainable Transitions: A Housing Crisis Case Study**

**Elizabeth Migoni Alejandre<sup>1</sup>, Alexander Wandl<sup>2</sup> and Arjan van Timmeren<sup>2</sup>, (1)Urbanism-ETD, Leiden University, Netherlands, (2)Technical University Delft, Netherlands**

The Netherlands, among many other countries in Europe, is facing a housing crisis, with an estimated amount of over 900,000 houses needed by the end of 2030 to satisfy short-term and increased urbanization demands. Governmental efforts are requiring input from universities, housing associations and other relevant stakeholders that can help guide the transition towards sustainable cities. These strategies are being developed, considering advances on construction processes and biomaterials that present positive opportunities for carbon and energy neutral buildings. However, in the middle of this transition towards environmentally sustainable cities, several questions remain on the underlying conditions that seem to be at the root of some of the problems we are trying to solve. The limited availability of trained human resources on the construction sector is presenting complications for the realization of projects, with a similar problem found on the material supply side, where communities that own extensively managed forest plantations are running out of people to work in them. Several factors are playing a role, among them, generational interests driven by consumerism and separation from the natural world by technocratic views surface as some of the most challenging to address. Furthermore, the high demand for housing is emerging not solely out of an increased global population and refugee crisis due to global political instability, but also due to high immigration to cities from rural areas. This increased urbanization seems to be influenced by both, a perceived lack of opportunities in rural regions, and as a direct result of the degradation of natural resources and landscapes, such as pollution, erosion, desertification, among many other impacts. Therefore, when addressing sustainable strategies, the question remains: are we asking the right questions? Interdisciplinarity thus, becomes essential for a robust assessment of the environmental implications of new design and service systems, challenging the current status with input from multiple stakeholders willing to redefine baselines. For researchers working on the development of global population scenarios and the quantification of environmental impacts, the challenge is to consider impact drivers that arise, for example, from socioeconomic differences and unsustainable societal trends, and work in collaboration with multiple disciplines for the development of integrative solutions.

#### **7.02.P-Mo404 Machine Learning Analysis of Regional Sustainable Development**

**Chen I-Chun<sup>1</sup>, Kui-Hao Chang<sup>2</sup>, Ming-Chin Kao<sup>2</sup>, Yen-Fu Liu<sup>2</sup> and Yang Chen<sup>2</sup>, (1)Department of Land Resources, Chinese Culture University, Taiwan, (2)National Cheng Kung University**

Based on the responsibility of sustainable development, the Taiwan government announced the Sustainable Development Goals (SDGs) in 2018, including 18 core goals and 143 sub-indicators to adopt the localization. The establishment pathway was only considered through a significant definition but the causal relationship between each indicator has been ignored. In addition, the changes in the sustainability of the regional governance need objective analysis. In view of this, we obtain the Taiwan open data, containing high information granularity at township levels, such as SDG1: no poverty; SDG2: zero hunger; SDG3: health; SDG4: education; SDG6: environment protection; SDG7: affordable energy; SDG8: economy; SDG11: sustainable city; SDG15 life below the land. To assess the cluster correlations and geographical characteristics of sustainability of the regional governance, we use the unsupervised machine learning methods to predict the spatiotemporal changes. Hopefully, the preliminary study's findings in the Kaohsiung city can apply the adaptation strategy for the sustainability of regional governance.

#### **7.02.P-Mo405 Using iSafeRat® Desktop as a Quick and Efficient Tool as Part of a Process to Develop New Safe and Sustainable by Design Substances**

**Gaspard Levet, Franklin Bauer, Floriane Larras, Etienne Bourgart and Paul Thomas, KREATiS, France**

In accordance with the EU Commission's desire to move to Safe & Sustainable by Design (SSbD) products, it is essential to develop new molecules that are both functional and as non-hazardous as possible. To help reach this goal, KREATiS proposes an innovative approach based on new molecule design optimization for use in chemistry, pharmaceuticals, cosmetics or agrochemicals. Using the Mechanism of Toxic Action (MechaA) of the molecules (predicted by MechaA+ profiler in the OECD

QSAR Toolbox or MechoA Premium model in iSafeRat® Desktop), we propose a tool that can aid R&D development, as part of the SSbD process, to help limit (eco)toxicological concern from early stages of product development, prior to performing regulatory studies and dossier submission. A workflow developed by KREATiS, has been successfully applied for several compounds in the fragrances, general chemicals and agrochemical industries. It can identify critical endpoints (e.g. unexpected MechoA, high toxicity to a given species) for the investigated molecules. Then, based on expert judgment, it is possible to provide clues on how to adjust the structure to avoid these, while trying to preserve the intended properties of the chemical (e.g. herbicidal action). The broad range of models included in iSafeRat® Desktop allows the identification of unwanted mechanisms of toxic action as well as the quantification of potential toxic effects before and during the development of new lead compounds. This workflow is a holistic approach, it uses a combination of different in-house models based on MechoAs, such as high-accuracy QSAR models predicting acute and chronic aquatic toxicity, an autoxidation profiler, sensitisation profiler, skin and eye irritation/corrosion models. Using predictive tools to estimate the relative toxicity of a large batch of promising compounds in early-stage research can save time and help focus on the most appropriate compounds. It is cheaper than experimental tests and respects the 3Rs paradigm. It also reduces the risk of problems later in the regulatory process. Combining *in silico* with key experimental tests can further increase confidence in the approach.

#### **7.02.P-Mo406 Including Criteria for GREENER Active Pharmaceutical Ingredients in the R&D Process: Opportunities and Need for Tools and Assays**

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Recently, we published criteria for GREENER active pharmaceutical ingredients. Application of this GREENER approach to drug discovery and development will help to reduce impact of medicinal residues on the environment. The criteria include effect reduction by avoiding non-target effects or undesirable moieties, exposure reduction via lower emissions or environmental (bio)degradability, no PBT (persistent, bioaccumulative, as well as toxic) substances, and, in those cases where it was not possible to apply these criteria, risk mitigation. With all these criteria however, patient health is of primary importance as medicines are required to be safe and efficacious in treating the disease. Before the criteria can be applied, tools and assays need to be developed in alignment with Drug Discovery and development principles. We will discuss which characteristics of these tools and assays are important and how current ecotoxicological tools may be adapted.

#### **7.02.P-Mo407 Environmental Impact of Pharmaceutical Products – A Systematic Review of Life Cycle Assessment Methods and Outcomes**

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During the life cycle stages of healthcare processes and products, many emissions and resource extractions occur that can have negative health and environmental effects. If one person is cured today, at the expense of making other people sick tomorrow, healthcare becomes counter-productive. Safe and sustainable pharmaceuticals can contribute to a healthy healthcare system. To understand the environmental consequences of pharmaceuticals, Life Cycle Assessments (LCAs) can provide valuable input. However, there is currently no agreement on the application and usability of pharmaceutical LCAs in footprint studies for the healthcare sector.

This literature review on all available scientifically published LCAs of pharmaceutical products until May 2022 aims to A) present the current state of the science of life cycle inventories (LCIs) and LCAs of pharmaceutical products whereby methodological choices, knowledge gaps and challenges are identified and B) present how to address these challenges in further research in the field.

LCAs of 63 pharmaceutical products with a known active pharmaceutical ingredient (API) and 6 with an unknown API have been retrieved during literature screening. The review also includes production processes such as solvent use (25 products), nuclei for API synthesis (2) and drug packaging (5).

Preliminary results show that common inconsistencies in performing LCAs for pharmaceutical products are missing life cycle stages, unspecified inventories, a limited life cycle impact assessment and missing interpretation steps. During the presentation, perspectives will be presented on how to address these challenges.

#### **7.02.P-Mo408 Recombinant Production of Peptide Antibiotics – An Interdisciplinary Approach to Develop Sustainable Antibiotic Alternatives**

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The ubiquitous contamination of the aquatic environment with antibiotic residuals accelerates the worldwide spread of antibiotic-resistance genes. Still, it is not predictable, how great the long-term impact of antibiotic-polluted surface and drinking water on human health will be. To face this global challenge, new effective, and rapidly and completely degradable antibiotics are urgently needed. An alternative to conventional antibiotics are natural structure-based antimicrobial peptides (AMPs).

The clinical application of AMPs requires an economic large-scale production process. The recombinant expression overcomes several drawbacks of chemical synthesis, but toxic side effects of the AMP on the host system request innovative approaches. A possible strategy is the expression and secretion of an inactive AMP-fusion protein.

For the biotechnological production, a modified sequence of the membrane active region of porcine NK-lysin is chosen.

Recombinant *Pichia pastoris* cells are cultivated in a highly instrumented bioreactor using an automated feeding strategy to reach high cell densities for maximum product yield. During purification, the fusion protein is captured by affinity chromatography, further, the final product is separated from its fusion partner. In the end, the activity of the *P. pastoris*-derived AMP is tested.

Analysis of the cultivation supernatant demonstrated that the fusion protein is secreted into the cultivation media, indicating that the expression system is not affected by its product. Activity tests showed that the purified AMP has a certain antimicrobial activity. In summary, the first results suggest that the applied strategy could be a promising approach to establish an efficient production process.

The success of heterologous large-scale production of AMPs will have a great impact on the reduction of the emission of antibiotics to the aquatic environment and the future availability of potent antimicrobial pharmaceuticals.

#### **7.02.P-Mo409 Sustainable by Design: An Aquatic Ecosystem Friendlier Alternative for Ciprofloxacin**

**Qiyun Zhang** and Karel De Schampheleere, Ghent University - GhEnToxLab, Belgium

The wide application of antibiotics has brought threats to freshwater ecosystems. Besides direct adverse effects on non-target aquatic organisms, the residues of persistent antibiotics in surface water enhances the selection pressure of antibiotic resistance (ABR) genes, too. The Safe and Sustainable by Design (SSbD) concept promotes sustainable pharmaceuticals that have lower potential hazardous impact on the environment throughout their life cycle: production, emission, degradation, and mitigation. An alternative compound derived from ciprofloxacin (CIP), CIP-Hemi, was developed based on the SSbD concept. Being higher in degradability than CIP, the chemical is likely to be less persistent in the environment. This study compared the ecotoxicity of CIP-Hemi and CIP using a cyanobacteria growth inhibition test. A species sensitive to CIP, *Microcystis aeruginosa* PCC 7806 was employed. As both CIP and CIP-Hemi are ionizable compounds, the test was performed at pH 8.0, at which the highest ecotoxicity of CIP is expected. Test results show that the observed ecotoxicity of CIP-Hemi, described by the 50% effect concentration (EC50), is at least 1 magnitude lower than that of CIP. Hence, CIP-Hemi is a promising compound that might result in reduced ecological risk to aquatic ecosystem.

#### **7.02.P-Mo410 Circular-Economy Challenge – The Power Of Rapid Screening of Chemicals in Plastics is Limited**

**Helene Wiesinger<sup>1</sup>**, Zhanyun Wang<sup>2</sup>, Verena Christen<sup>3</sup>, Miriam Langer<sup>3</sup>, Roxane Pasquettaz<sup>4</sup>, Christophe Bleuler<sup>4</sup>, Philippe Favreau<sup>4</sup> and Stefanie Hellweg<sup>1</sup>, (1)ETH Zürich, Switzerland, (2)Swiss Federal Laboratories for Materials Science and Technology (EMPA), Switzerland, (3)University of Applied Sciences and Arts Northwestern Switzerland - FHNW, Switzerland, (4)Service de l'air, du bruit et des rayonnements non ionisants - SABRA, Switzerland

Plastic recycling is urgently needed to mitigate climate change and other environmental impacts associated with the production of virgin plastics. However, plastics can contain various chemical substances, including residual monomers, additives, processing aids and non-intentionally added substances (NIAS). These chemical substances can pose various challenges to the transition to a circular economy: by polluting secondary materials, interfering with the recycling process, or having negative health impacts on consumers and workers. Currently, manufacturers provide little information up-front on which substances are present in a specific product, rendering rapid screening of chemicals as a necessary alternative to provide this information. In this study, we aimed to find a suitable, simple and fast screening method to determine which plastic products may contain chemicals posing a high risk to consumers or the transition to a circular economy. We employed complementary techniques to rapidly screen polyvinyl chloride (PVC) building materials for specific chemicals and in-vitro biological effects. Specifically, we used XRF for elemental analysis, solvent extraction and gas chromatography–mass spectrometry (GC-MS) for suspect screening, and solvent extraction and four bioassays (i.e., MTT, Reactive Oxygen Species (ROS), Yeast Estrogen/Androgen Screen (YES/YAS), AMES) for biological screening. We found that many of the building-material samples contained toxic metals (mainly lead), restricted phthalates (mainly DEHP), alternative phthalates (mainly DiNP and DiDP), and alternative plasticizers (mainly DEHT and DEHA), whereas a few samples contained phosphor flame retardants. Some samples showed activity in the employed bioassays. None of the employed methods could predict the presence of problematic substances nor the biological activity of samples on its own. This suggests that a host of methods is needed to get a full picture of the chemical content in a product, and thus, its fitness for recycling. Owing to the immense workload associated with such comprehensive screening campaigns, even the most ambitious efforts will not be sufficient to ensure, in a timely and cost-effective manner, that all hazardous chemicals are eliminated from our material cycles before recycling. Therefore, concerted action is urgently needed to ensure up-front transparency of chemicals in plastics.

#### **7.02.P-Mo411 Safe, Sustainable and Circular by Design Organophosphate Flame Retardants**

**Hannah Flerlage<sup>1</sup>**, Bianca Stadelmann<sup>1,2</sup>, Michiel Kraak<sup>3</sup>, Antonia Praetorius<sup>3</sup> and Chris Slootweg<sup>1</sup>, (1)Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Netherlands, (2)University of Amsterdam, Netherlands, (3)University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics, Netherlands

Safe and Sustainable by Design has gained traction in research and policy spheres as a key tool to achieve a non-toxic environment. However, there are limited examples of the practical application of this concept. In this work we present a case study of redesigning the flame retardants tris(2-chloroethyl)phosphate (TCEP), listed as substance of very high concern under REACH due to its toxic effects on reproduction, and its analogue tris(3-chloropropyl)phosphate (TCPP), for which less data is



available. Both chemicals are frequently found in environmental monitoring studies. The central aim of our redesign is to change the molecular structure to reduce hazard.

We build on a computer-aided approach using *in silico* generation of chemical structures similar to the original chemical and available, established, and applicable QSAR models to predict PBMT related properties. Multicriteria analysis ranked generated structures according to predictions and inspection of the top-scoring compounds identified promising structural features to inform our design. Elements of sustainable production were also considered. Retrosynthetic fragments with a high potential to be sourced sustainably in the future are chosen. Regarding the phosphate core of the molecule, research is underway in our labs to synthesize circular organophosphates from phosphates recovered from wastewater.

We propose two alternative chemicals for TCEP and TCPP, respectively. With a straight-forward design choice we strive to give a clear-cut example of the feasibility of tuning environmental properties by molecular design. The poster will present the redesign process including results of a suite of experimental tests comparing the environmental safety of the original compounds TCEP and TCPP, with that of the newly designed compounds. Experiments are primarily focused on the aquatic environment as the expected most affected compartment exposed via wastewaters. Two experiments are run to study biodegradability, (1) assessing mineralization via CO<sub>2</sub> evolution in different media (sewage sludge, freshwater) using a respirometry system, and (2) studying primary degradation and transformation products using LC-MS/MS. Acute and chronic ecotoxic effects on algae, daphnia, and chironomids are determined. Furthermore, we will test the function of the alternatives in flame retardancy tests. This extensive series of experiments will likely make a solid case demonstrating the feasibility of Safe and Sustainable by Design.

#### **7.02.P-Mo412 A 2-Phase Methodology to Assess the Environmental Implications of Agricultural Systems to Inform Sustainability Improvements: Conservation Agriculture as Example**

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To improve sustainability of farming it is important to understand the environmental costs and the benefits of the various tools and methods that are applied within diverse cropping systems.

Based on the available peer-reviewed scientific literature, we initially developed a methodology that used the environmental categories that are usually addressed within environmental risk assessment processes for plant protection products as starting point, and applied it to various other, non-chemical tools that are commonly part of agriculture. Finally, a range of abiotic and biotic impact categories were considered, featuring 12 assessed categories for soil, water, climate and biodiversity.

In a second phase of the work, we explored the scientific literature about the environmental implications of the production systems where those tools and methods are applied. We took the example of conservation agriculture with a focus on wheat production in temperate climates (e.g. comparable to Central Europe) to explore the environmental benefits and limitations of that specific system.

The results of phase 1 show a nuanced picture of the environmental implications related to direct impacts, indirect impacts, their selectivity and potential data gaps when it comes to the different tools and impact categories. Concerning phase 2, for conservation agriculture a multitude of environmental benefits were found, although in temperate climates the system may also present several limitations.

The two-phase assessment of the different tools and environmental impacts of the systems was able to create a broad and nuanced perspective to identify opportunities and trade-offs. This combined approach can therefore help to build a better and more holistic understanding of the agronomic and environmental implications of different management options. The farmer then has the potential to use this information to improve the overall sustainability of their production system, placing emphasis on the environmental impacts that are most important in their particular location and within their agricultural system.

In addition, this approach can support policy-makers to facilitate decision-making by taking into account the available scientific evidence and its conclusiveness, thereby also increasing transparency towards the public and various other stakeholders.

#### **7.03.P Bioelectrochemical Systems Technology for a Better Environment**

##### **7.03.P-Mo413 Microbial Electrochemical Systems to Remove Bioactive Pollutants From Water: Finding the Nexus Between Composition of Anodic Microbial Communities, Removal Efficiency and Electrochemical Performance**

*Claudio Avignone Rossa, Razieh Rafieenia, Mira Sulonen and Daniel Farkas, Department of Microbial Sciences, University of Surrey, United Kingdom*

The release of pollutants to the environment, caused by human activity, contaminate soils and water bodies with a diverse collection of biologically active compounds, including antibiotics, endocrine disruptors, pharmaceutical and personal care products, pesticides, and other types of potentially toxic molecules, such as chlorinated compounds, polycyclic aromatic hydrocarbons, etc. Some of those compounds are very difficult to remove from water using conventional methods, and remain at concentrations hazardous for humans, animals and plants.

In recent years, developments in microbial electrochemical systems (MES) have prompted their application in wastewater treatment systems. In devices called microbial fuel cells (MFC), electroactive microorganisms colonize the anode, using it as the terminal acceptor of electrons released in the catabolism of substrates present in the feedstock. The circulation of those electrons through an external circuit towards a cathode generates an electrical current. If electroactive microorganisms (single species or microbial communities) are able to degrade those pollutants, they can be inoculated in the MFC, and the combination of their metabolic capabilities with their capacity for extracellular electron transfer may result in efficient removal of recalcitrant pollutants.

In this talk, I will discuss advances in the design of MFCs to overcome the limitations of the process, and how the microbial communities can be studied and analysed to steer their metabolic and electrical performance towards higher process efficiencies. I will present examples of recent work for removal of pesticides, antibiotics, hydrocarbons, and heavy metals from polluted water of diverse origins, and show how the composition of the microbial communities involved can be manipulated to improve their activities.

### **7.03.P-Mo414 Studying the Extracellular Electron Transfer Mechanisms of *Desulfuromonas acetoxidans* to Develop Sustainable Energy Production and Water Desalination**

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One of the major sustainability challenges of our time is to provide fresh water for human consumption and activities in a scenario of increasing demand from a rapidly growing human population. Since 97% of the planet's water supply is in the oceans, water desalination technologies have been emerging as a solution to tackle this major problem. Microbial Desalination Cells (MDC) have the advantageous particularity of producing potable water in a combined process of producing electricity from wastewater treatment. In MDC, electroactive bacteria act as catalysts by supplying this bioelectrochemical system with electrons from the oxidation of organic matter that can be found in wastewaters. Since most characterized electroactive bacteria are not tolerant to high salt concentrations, MDC are inherently prone to be energy inefficient and not suitable for saltwater desalination. In order to improve and optimise MDC technology, we focused our attention on the marine and electroactive bacterium *Desulfuromonas acetoxidans* that has been shown to be prevalent in bioanodes operating in conditions of high salinity. Our work aims to evaluate *Desulfuromonas acetoxidans* DSM 684 electroactivity using *in vivo* and *in vitro* studies. The capacity of *Desulfuromonas acetoxidans* to tolerate salt and produce current in a bioelectrochemical system was assessed, revealing that the optimal growth of *D. acetoxidans* is at 2% NaCl, while current production did not show significant difference between 1, 2 and 3%. In order to understand the key molecular players in the current production, a putative electron transfer pathway composed by multiheme cytochromes was identified in *D. acetoxidans* compared with other related electroactive organisms and characterized using *in vitro* studies. Altogether this study constitutes the template for the better understanding of the pathways of *D. acetoxidans* that are key for its application in MDC towards sustainable energy production and water desalination.

### **7.03.P-Mo415 Bioelectrochemical Treatment Wetlands for Safeguarding Wastewater-borne Antibiotic Emissions**

Vaidotas Kisieliūsis<sup>1</sup>, René Kilian<sup>2</sup>, Jens C Pors<sup>2</sup>, Carlos Alberto Arias<sup>3</sup> and **Pedro N Carvalho<sup>1</sup>**, (1)Environmental Science, Aarhus University, Denmark, (2)Kilian Water ApS, Denmark, (3)Aarhus University, Denmark

Concerns on the occurrence of aquatic pollutants, specifically, antibiotics, antimicrobial resistant microorganisms and microbial pathogens in the environment is increasing. This in turn increases the attention on the performance of wastewater treatment technology for these types of pollutants.

Wastewater is considered a major source of emerging contaminations emission to the environment, mostly because traditional technology and existing treatment plants are not designed to address these pollutants. As a result, efforts to mainstream nature-based solutions to manage wastewater need to carefully assess their performance in relation to antibiotics, antimicrobial resistant microorganisms and microbial pathogens.

The current work, being developed under the umbrella of Project Nature, aims at assessing the performance of a novel hybrid technology that merges a bioelectrochemical system with a treatment wetland. More specifically, this novel technology is being benchmarked against current state-of-the-art treatment wetland technology (vertical flow and intensified with aeration) for secondary wastewater treatment.

Three full-scale systems (30 - 200 PE) implemented in Denmark treating domestic wastewater are being monitored since May 2022 for 1-year period. Influent and effluent are being sampled monthly and analysed for target model antimicrobial compounds and respective transformation products, and DNA-based techniques to characterize pathogens and antimicrobial resistance, together with classical water quality parameters. The selected list of target compounds includes 14 antibiotics (e.g., azithromycin, erythromycin, sulfadiazine); 5 antimicrobials (e.g., quaternary ammonium chlorides), 3 antifungal agents (e.g., miconazole), and over 30 other domestic wastewater-relevant micropollutants.

The first results indicate that several antibiotics are measured in the influent of the study sites. However, it can be seen that the decentralized wastewater systems (< 200 PE) show different antibiotic occurrence patterns and concentrations than in large urban wastewater treatment plants. Removals above 63% (of e.g. trimethoprim) have been observed.

At SETAC Europe we will present the first monitoring results of the bioelectrochemical treatment wetland, a unique system in Scandinavia, with a large potential for replication. The potential of these nature-based solutions to control antimicrobial emissions will be very important for their future application.

### **7.03.P-Mo416 Electrostatic Separation of Nanoplastics from Wastewater**

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Microplastics, generated by the degradation of plastic waste, have been found in almost all ecosystems across the globe and remained catching attention as an emergent pollutant. Such micron-sized particles, however, may only be the tip of the iceberg. Nanoplastics, represent the nanoscale size of these tiny particles. With sizes much smaller than 1 µm, they can be even more environmentally problematic as such particles may be taken up and interfere with individual cells of organisms. Still, their very small size makes identification and removal much more challenging compared to conventional microplastics. Thus, the research

field of Nano plastics is still in its infancy. In our present work, we aim to remove Nanoplastic materials from wastewater through the use of their unique properties. We apply a combination of electrochemical and colloidal processes to remove such tiny particles from an aqueous phase. A model system has been chosen as a starting point to have a control over all the influencing parameters and to prove a basic understanding of the separation mechanism. These findings are consequently transferred to industrial wastewater as a real environmental system. Both systems have been efficiently treated and cleaned from nanoparticles. The recovery percentage reached 98% and 95% from the model system and the industrial wastewater systems respectively. To take the process further, a scale up of the technique in a continuous mode is currently being performed.

#### **7.03.P-Mo417 Bioelectricity Production by Terrestrial Microbial Fuel Cells using a PAH Contaminated River Sediment**

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Dredged river sediments can be contaminated by Polycyclic Aromatic Hydrocarbons (PAHs) due to their accumulation in sediments.

Terrestrial Microbial fuel cells (TMFCs) are bioelectrochemical system with soil or sediment as the electrolyte. TMFCs have been investigating for their ability to harvest electricity from microbial activity and, at the same time, biodegrading persistent contaminants.

In this framework, a TMFC experiment in open and closed-circuit conditions was set up with a river sediment collected from a navigable channel (Bega) located between Romania and Serbia. It contained PAHs (920±153 µg/kg), total petroleum hydrocarbons (1799±286 mg/kg) and heavy metals (Cr, Cu, Pb and Zn). Some TMFCs were also set up with sediment plus compost (3% w/w) for testing if an additional carbon source could enhance microbial activity. Control sediment (without cathode and anode) were also set up. TMFC polarization curves were performed daily by a measurement station. The circuit was closed applying several resistances.

TMFC activation time was relatively low, probably due to the microbial community adaptation to contaminant presence. Compost addition had a beneficial effect on the power output (maximum average power density: 173 mW/m<sup>2</sup> in compost presence; 53 mW/m<sup>2</sup> in compost absence). These values were highlighted by the internal resistance behavior. In fact, TMFCs with compost recorded an initial lower external resistance (300 Ω) if compared to sediment without compost (1000 Ω). The resistance reduction was due to the conductivity increase, probably for microorganism proliferation. At the end of the experiment (4 months), anode, bulk and cathode samples were collected for microbiological, ecotoxicological and chemical analyses. Total microbial number and activity were analyzed and the *Allivibrio fischeri* and *Heterocypris incongruens* ecotoxicological tests were performed for all conditions. Moreover, PAH (EPA 8000b), TPH (ISO16703:2004) and heavy metals (EPA7010) were analysed. Finally, a spectroscopic characterization of sediments was performed by an ASD FieldSpec portable spectroradiometer. Microbiological analyses showed the presence of more active bacterial populations in compost presence, with the highest values at anode. The ecotoxicological tests showed some positive effects on the organisms tested presumably due to the non-complete PAH degradation, with the formation of toxic transformation products.

#### **7.03.P-Mo418 Bioelectrochemical Systems as a Platform for Biodegradation and Biosynthesis of Surfactants**

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Microbial fuel cells (MFCs) have been recognized as systems that are capable of degrading multiple types of pollutants, including problematic hydrophobic compounds. In recent years, significant efforts have been made toward MFC-based degradation. Such an approach may become a solution for both in-situ and ex-situ methods of biodegradation. MFCs and other types of bioelectrochemical systems (BESs) give the unique opportunity to control the bacterial metabolism through electrical circuits, as well as to extract energy from degraded organic matter.

In this work, petroleum and waste cooking oil degraders and biosurfactant producers were acquired from several types of environments. These communities were sampled over Europe and Asia, including: pristine environments, moderately contaminated nature areas, urban environments, and heavily contaminated sites. Small scale MFC and BES designs, were used to adapt and develop communities capable of degrading hydrophobic waste products. These communities were further investigated towards biosynthesis of surfactants. The waste cooking oil as fuel revealed higher surface activity, reaching 37 mN/m, while the occurrence of surface activity for petroleum was recorded only after several months of adaptation and reached 47 mN/m. In both cases, the occurrence of biosurfactants was correlated with the electrochemical activity, suggesting a direct contribution of biosurfactants in metabolism in MFC-supported biodegradation.

The analytical studies revealed the presence of rhamnolipids based on both types of substrates as well as microbial species producing other types of biosurfactants. Therefore, we have shown that biosurfactants can be produced within BES systems to combat the environmental pollution.

#### **7.04 Environmental Toxicology and Chemistry in Africa: Tackling Legacy and Emerging Pollutants**

#### **7.04.P-Mo420 Evaluating the Potential Human Health Effects and Impact of Gold Mine Activities on River Osun, Osun State Nigeria**

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The quest for safe potable water is increasingly required by the day most especially in developing countries globally. Surface water is one of the main sources of water supply and this has contributed significantly to the larger population groups for both domestic, agricultural, and in some cases industrial use. Osun River is a major river within its catchment area that flows southwards through central Yorubaland in southwestern Nigeria into the Lagos Lagoon and the Atlantic Gulf of Guinea. It is sacred and reserved along its course within the Osogbo groove. Human activities such as mining practices by illegal and unregulated licensed gold mining activities at the upper course along the Ilesha axes have caused a sudden change in the colour of the river thereby influenced negatively, compromising the quality of the Osun River. Therefore, there is a need for urgent attention to know the current state of this river for the health and safety of people that depend on it. In this study, the impact of mining activities with the probable health risk associated with precious metals such as gold and silver as well as other selected heavy metals were collected at six (6) strategic points along the course of this river. A 250 mL water sample was collected in sterilized screw-capped amber plastic bottles at a depth of 10-15 cm from the flowing river at each point following a standard method (Harmel et al. 2010). Water samples were acidified using nitric acid. The samples were filtered using 0.45 µm Acrodisc syringe filters, prior to analysis. The selected heavy metals were analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer, Waltham, Massachusetts, United States) at a selected wavelength. The result indicated that the metals investigated were found to be present in the samples along the river course. The concentrations of the metals ranged from 33.40 – 137.0 µg/L (Au), 33.30 – 434.0 µg/L (Ag), 231.80 – 405.20 µg/L (As), ND (Cd), 58.80 – 71.20 µg/L (Co), 78.80 – 168.20 µg/L (Cu), 1330 – 3880 µg/L (Fe), 85.80 – 246.30 µg/L (Mn), 58.0 – 96.10 µg/L (Ni), 35.40 – 97.10 µg/L (Pb) and 120.70 – 428.00 µg/L (Zn). All the heavy metals were present except Cd which was below the detection limit. Most of the heavy metals investigated could likely pose adverse health effects to the aquatic life and the humans that get exposed to this river.

#### **7.04.T-01 Utilizing a Geographic Information System-based Knowledge Hub for Contaminants of Emerging Concern in South African Water Resources developed using Open-Source Software**

**Tarryn Lee Botha, Water Research Group, North-West University, South Africa**

Protecting surface freshwater sources is now a top priority in water resource management due to population increase and diminishing resources. In the past, society has responded to issues based on the availability of financing, the gravity of the threat, and the level of public outrage. The ability to identify the causes of contaminated water supplies is crucial for achieving this. These elements are continually shifting as new contaminants are released into sources of surface water. The interest in contaminants of emergent concern (CEC) is growing as we go deeper into the digital age. Unrestrained pollutants are contaminants of emerging significance because they are now being found in our water bodies. These can include nanomaterials, flame-retardants, microplastics, agricultural waste, pharmaceuticals and personal care products, which may cause ecological or human health impacts. The continued unregulated use of these products could lead to further ecological risks. Although studies are being done to determine which contaminants are present in South African water sources, the results of these studies and the information that is currently available are not compiled and presented to the scientific community and stakeholders in readily accessible formats and platforms. To create environmental regulations, authorities must be informed of recent study findings. With the aid of technology from the fourth industrial revolution, we were able to compile data from the literature and present it to both regulatory authorities and researchers in an accessible geographic online format. In order to enable visualization on an interactive map that can be continuously updated, a standard Excel spreadsheet was created, uploaded to a PostgreSQL database running a PostGIS extension, and then processed in the GeoServer. The ability to access information in near-real time will lessen the likelihood of redundant research efforts, foster interdisciplinary cooperation, and serve as a CEC early warning system for South African water resources. This technology can be expanded upon to include several other geographical regions.

#### **7.04.T-02 Toxic Informal Solar Lead-Acid Battery Recycling in Malawi**

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In Malawi, electricity deprivation is recognised as a risk to the country's development and future stability. With the inadequacy of the national electricity grid, Malawi's national electrification strategy depends on the private off-grid solar market, aiming to import and deploy millions of household scale off-grid solar (OGS) products by 2030, currently in absence of a waste management strategy. Malawi's OGS market is predominately unregulated; solar home systems are distributed by unmonitored and uncertified suppliers and rely on lead-acid batteries with short expected lifetimes (< 4 years). This study investigates the informal recycling of lead-acid batteries in Malawi, which is known to be one of the world's most toxic industries but has not been reported in Malawi before and has not been considered in relation to electrification strategies across sub-Saharan Africa. Data was collected by observing two informal recyclers in peri-urban villages near the capital city of Lilongwe carrying out the informal recycling process for lead-acid batteries used in off-grid solar home systems. The data was collected as part of a series of studies addressing off-grid solar waste in Malawi, including a mass flow analysis of the informal lead-acid battery recycling process. The rudimentary methods of fabricating improvised lead battery cells are described, slightly differing between the recyclers but following the same principles.

Between 3.4-4.2 kg of lead was lost to the environment during the informal recycling of a single battery (0.27-0.29 kg lead lost per kg of battery), representing 108-133 times the oral lethal dose of lead for a 70 kg adult. Either 0.67 or 3.66 kg of dilute sulphuric acid was split to the floor (0.05 of 0.25 kg sulphuric acid lost per kg of battery), depending on if the acid was collected

and reused in the recycled battery or discarded on the street. There is a paucity of research on the lead exposure and absorption pathways from informal lead-acid battery recycling in low- and middle-income countries, although, the observed battery recycling processes took place on busy streets within peri-urban villages, exposing densely populated communities to life-threatening quantities of lead pollution. These severe environmental and human health burdens risk being exacerbated by Malawi's national OGS electrification strategy. Further studies are urgently needed to investigate the health impacts of OGS waste across sub-Saharan Africa.

#### **7.04.T-03 Examining the Impact of Pyrethroid Leaching From Insecticide Treated Net Fishing on Mortality and Oxidative Stress Biomarkers in Aquatic Organisms**

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The distribution of insecticide treated mosquito nets (ITNs) is a great public health success story, with studies suggesting these nets reduced the global burden of Malaria by approximately 40%. ITNs are typically treated with pyrethroids, which are known to have a low toxicity to mammals and high toxicity to aquatic organisms. Recent studies have shown that ITNs are being used for alternative practices, including fishing. In a study from a waterside community located on Lake Tanganyika, 87.2% of people utilized their mosquito net for fishing as opposed to malaria protection. Coinciding with reports of off label fishing, there are reports of a decrease in fish quality and quantity. Quantitative data is missing to support these claims, so we designed experiments in both vertebrates, *P. promelas*, and invertebrates, *D. magna* to investigate the toxicity of ITNs. Organisms were exposed to varying sizes of nets, 1cm<sup>2</sup>, 5cm<sup>2</sup>, 10cm<sup>2</sup>, 20cm<sup>2</sup>, and non-treated 20cm<sup>2</sup> sized nets. Two pyrethroid treated nets were tested alpha-cypermethrin treated nets and deltamethrin treated nets. We tested six exposure scenarios for 7 days and mortality was recorded. To test for sub-lethal effects, we focused on oxidative stress endpoints that have been linked to pyrethroid exposure. Following sublethal exposures, we measured SOD1,2, Catalase, P53, PXR, and CYP3A4 expression using qPCR in the *P. promelas* larvae to expand our understanding of oxidative stress and sublethal impacts. Results from our mortality experiments showed that both ITNs used in our experiments are highly toxic to invertebrates. The use of new Alpha-cypermethrin treated net led to 100% mortality in all treatment groups in under 48 hours. When organisms were exposed for 30 minutes we observed 100% mortality in the 5cm<sup>2</sup>, 10cm<sup>2</sup>, and 20cm<sup>2</sup> sized nets and a slight reduction in toxicity for the 1cm<sup>2</sup> net. For our Deltamethrin experiments, using a net new all treatments reach 100% mortality by 72 hours. When we reused the nets throughout the experiment, all treatment groups except for 1cm<sup>2</sup> reach 100% mortality by 168 hours. When the organisms were exposed for 30 minutes, all treatments reached 100% mortality, except for the 1cm<sup>2</sup> sized net which reached 40% mortality. Results from our sub-lethal exposures include significant results for all of our genes when compared to our housekeeping gene. Results from these studies indicated that ITNs are highly toxic to invertebrates and can lead to sub-lethal changes following exposure.

#### **7.04.T-04 Air pollution in Dakar (Senegal): A Case Study on Outdoor and Indoor Exposure**

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The harmful effects of indoor and outdoor air pollution on human health are of great concern nowadays in several regions of the world. Dakar, the capital and biggest city of Senegal, is characterized by a vehicle fleet characterized by poorly efficient combustion technologies that largely contributes to the deterioration of air quality. In this study, we firstly evaluated indoor and outdoor air pollution levels (CO, CO<sub>2</sub>, NO, NO<sub>2</sub>, PM<sub>10</sub>) at an urban site in Dakar city center and at a rural site. Then, the individual exposure levels to selected pollutants and the variations in the levels of biomarkers of exposure were investigated in different groups of persons (bus drivers, traders working along the main roads and housemaids).

BTEX analysis revealed that toluene was the volatile pollutant with higher concentrations, ranging from 800 to 1950 µg/m<sup>3</sup>.

Benzene and xylenes concentrations were from 150 to 650 µg/m<sup>3</sup> while ethylbenzene was always around about 50 µg/m<sup>3</sup>.

Benzene concentration was significantly higher for the housewives group than for professionals. The high concentrations found for housewives is probably due to the cooking habits (coal used in kitchens as combustible), local practices (important incense burning) and use of cleaning products that are important emitters of volatile organic compounds. Different urinary metabolites were selected to assess human exposure. t-t-MA was not detected (below the detection limit) in urine samples, while S-PMA values were higher for housewives when compared to professionals. The level of 1-HOP, used to assess a pyrene exposure, was slightly higher in drivers (compared to traders, city housewives and district semirural housewives). Statistical analysis revealed that urinary 1-HOP levels were significantly higher for urban site housewives compared to those at the semirural district.

Moreover, urinary levels of DNA oxidative stress damage (8-OHdG) and inflammatory (interleukin-6 and -8) biomarkers were higher in urban subjects in comparison to rural ones. The air quality measurement campaign showed that the bus interior was more polluted with PM<sub>10</sub>, CO, CO<sub>2</sub> and NO than the market and urban or rural households. However, the interior of households showed higher concentration of VOCs than outdoor sites confirming previous observations of higher indoor individual exposure level to specific classes of pollutants.

#### **7.04.P Environmental Toxicology and Chemistry in Africa: Tackling Legacy and Emerging Pollutants**

#### 7.04.P-Mo421 Catalytic Ozonation of 4-chlorophenol using Beta-iron oxy hydroxide ( $\beta$ -FeOOH) Nanoparticles: Efficiency and Toxicity Studies

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Nano-based water treatment applications and processes are currently receiving much attention due to their perceived ability to resolve numerous challenges encountered during conventional water treatment operations. They provide innovative and progressive approaches to solve water scarcity, and contamination issues around the world. Despite their huge potential, the use of nanomaterials for water treatment purposes is raising concerns about the safety and health implications of our environment and biota therein.

The utilization of polymer matrices as support materials for the immobilization of nanomaterials can offer some measure of safety by preventing their release into the environment during process application, and also brings about ease of recovery and regeneration for reuse. This study focused on the “safe application” of the beta-iron oxy hydroxide ( $\beta$ -FeOOH) nanoparticles, in continuation of previous work that established its capability as an effective catalyst when applied in catalytic ozonation processes. The  $\beta$ -FeOOH nanoparticles were synthesized using the hydrothermal synthesis method and incorporated into polyamide matrix via the *in-situ* method to fabricate magnetic nanocomposites. The FTIR, TEM, SEM, EDS, PXRD and nitrogen adsorption-desorption characterization were carried out for the materials. Catalytic properties of the  $\beta$ -FeOOH/polyamide nanocomposite (1.25 wt%) was evaluated by assessing its ability to degrade 4-chlorophenol (4CP) in aqueous solutions through catalytic ozonation processes; samples were collected at different time intervals and analysed by LC-MS-TOF. The effect of pH on degradation efficiency of the 4CP was also evaluated. Results showed a 98.0 % degradation of 4CP within 40 min relative to 64.01 % for ozonation alone. The efficiencies of the nanocomposite for 4CP degradation were 85.36 %, 86.54 % and 95.05 % obtained for pH 3, pH 7 and pH 10 respectively at 30 min, with corresponding rate constants of 0.0645, 0.0665 and 0.1123. The composite exhibited excellent reuse potential with minimal decrease of its degradation ability over six cycles. No leaching of iron was observed when applied in acidic, neutral and alkaline conditions. Additionally, the COD and TOC values for real wastewater was effectively reduced by 51.28 % and 89.22 % after 60 min by the polymeric nanocatalyst. The *Daphnia* acute toxicity test results affirmed the need for ecological and human health risk assessment of remediated wastewater.

#### 7.04.P-Mo423 Comprehensive Monitoring of Surface Water in the QwaQwa Area (South Africa) Combining Chemical and Biological In-Vitro Tests

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QwaQwa, a former homeland area in South Africa, is part of the Free State province. Recently the area has been plagued by regular interruptions in water service delivery, mainly due to water scarcity, poor and aging infrastructure, poor water governance and corruption. The situation is exacerbated by poor surface water quality, putting local communities using the water for drinking and irrigation at high risk compromising. Despite this, information on the water quality and ecological condition of the local rivers as the main water resources, is very limited. A once-off sampling campaign was carried out in March 2022 to record the potential pollution of the surface waters. The results of this study will enable the local authorities to identify sources of pollution and to develop targeted measures. Towards this purpose, biological and chemical monitoring was carried out in this area at 4 rivers and with a total of 9 sampling sites. Our hypothesis that the contamination corresponds to countries with established wastewater treatment could not be confirmed. Beside monitoring physico-chemical parameters, effect monitoring with a total of 9 in-vitro tests together with extensive chemical analysis was carried out with the water samples. The results show a high pollution of water bodies in urban areas and a lower pollution of rural areas. For the effect monitoring the yeast estrogenic screen (YES) showed at two sampling sites in urban areas response and the yeast dioxin-like screen (YDS) responded at all sampling sites except the two sites closed to the minor populated source of the rivers. Similar pattern was found by chemical analyses. However, pharmaceuticals as micropollutants show a clear difference to developed regions, such as Europe, which correlates with age distribution as well as with occurring diseases. For example, HIV drugs were found in considerable concentrations as a special feature. Iron, zinc and aluminium were detected above the environmental quality standards, however the proof of origin was unclear. The detected pollutants corresponds to a general expected patterns for these areas, but in detail very specific evidences were also found, for example in the analyses of pharmaceuticals. For this area a decentralised management of water purification is proposed to solve water pollution issues.

#### 7.04.P-Mo424 Oxidative Stress Biomarkers of Metal Pollution in the Non-Native Macrophyte, *Ceratophyllum demersum*, Cape Town, South Africa

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Our research has shown that Cape Town's urban rivers are generally heavily polluted with metals, particularly Zn, Fe, Al and Cu. The Diep River, Milnerton, has been of particular interest to us, as it is one of the largest and most important freshwater systems in Cape Town, surrounded by numerous pollutant sources. The macrophyte *Ceratophyllum demersum* is known to be a hyperaccumulator of metals and used for phytoremediation. It grows prolifically in the Diep River. Our study investigated metal

toxicity in *C. demersum*, by determining chlorophyll content and antioxidant responses in plants collected from a heavily polluted river site and compared them with specimens growing in an unpolluted pond (reference plants), to determine whether these responses can be used as biomarkers of oxidative stress. Concentrations of Zn, Fe, Al and Cu were determined with an ICP-MS, whilst chlorophyll *a*, *b* and total chl were determined by spectrophotometry. A suite of antioxidant responses was investigated. River plants generally showed significantly ( $p < 0.05$ ) higher metal bioaccumulation. AsA, CAT, SOD and TBARS were all significantly higher in river plants, whilst TP, FRAP, ORAC and GSht were all significantly lower ( $p < 0.05$ ). CD's did not differ. Results showed that, although reference plants experienced some stress, river plants experienced far greater pollutant-induced oxidative stress. Despite this, the river plants did not show significantly lower chlorophyll content, demonstrating the species' ability to tolerate oxidative stress. When reference plants were transplanted into the polluted river in baskets and exposed for 12 weeks, they bioaccumulated metals to significantly higher concentrations ( $p < 0.05$ ) than the naturally occurring river plants and chlorophyll production was significantly promoted ( $p < 0.05$ ) during the 12 weeks, probably due to the additional nutrients in the polluted water. Our study showed that antioxidant responses in *C. demersum* seem to be reliable biomarkers of oxidative stress due to metal and pollutant-"cocktail" exposure in the field. However, chlorophyll content as field biomarker requires further investigation. We propose *C. demersum* as possible model in South African aquatic biomarker studies, due to its hyperaccumulation abilities, generally sensitive responses to chemical changes, high tolerance and abundance.

#### **7.04.P-Mo426 Speciation of Heavy Metals and Health Risk Analysis In Some Selected Agricultural Zones In Taraba State Nigeria**

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The soil environment is a reservoir of nutrients and pollutants to Plants and Animals. Several of these pollutants have been implicated in causing several health issues in both humans and wildlife species. Agricultural soil plays major role in food safety and security. Most of our food items are laden with lots of pollutants and the consumption of contaminated foods has serious implication on health. Heavy metals are potential environmental pollutants, they have the ability to bio-accumulate in plants and eventually get to humans through food. This study evaluated the presence of heavy metals (HM) and health risk assessment in soil on arable farmlands in selected agricultural zone of Taraba State, Nigeria. Different soil samples were collected and processed, HM (Pb, Cd, Cr, Hg, and As) concentrations were analyzed using Atomic Absorption Spectrophotometry (AAS). The results showed that Chromium concentration was generally high in all the studied areas with values ranging from 1.71 to 2.29mg/kg. However, Cadmium and Arsenic followed moderately with values ranging from 0.05 to 0.07mg/kg while Lead and Mercury had the lowest concentration of less than 0.03mg/kg across the studied areas. Ecological Risk Assessments parameters: Target Cancer risk, Hazard index (HI) and estimated daily intake (EDI) was determined to assess the non-carcinogenic health risk. Bali LGA recorded the highest levels of HM having the highest health risk, while Yororo and Zing LGAs had low hazard risk index. This Study shows that although heavy metals were present in the selected soil samples, the risk of exposure is low and may not pose any health challenge to humans based on the concentration detected.

#### **7.04.P-Mo428 Origin and Chemical Characterization of Atmospheric Pollution (PM<sub>2.5</sub>) in Dakar (Senegal)**

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9 million of deaths are attributable to air pollution, mostly particulate (i.e. PM), which refers to a complex mixture of airborne chemicals (Lelieveld et al. 2020). The objective of this study was to chemically characterize fine particles (PM<sub>2.5</sub>) sampled at Medina, a locality strongly influenced by road traffic, and to determine their natural and/or anthropogenic origin. To do so, 74 samples of PM<sub>2.5</sub> were collected (5 days a week and 10 h/day) using a high-speed cascade impactor (Staplex, model TFIA, New York, USA). These particles were subject to an organic chemical characterization (PAHs and paraffins) by gas phase chromatography coupled to mass spectrometry (GC-MS) and an inorganic analysis (elemental and ionic composition) by spectrometry of inductively coupled emission (ICP-OES) and ion chromatography (IC). On average, the annual emission of PM<sub>2.5</sub> at Medina is estimated at 152.7 µg.m<sup>3</sup>. 15 polycyclic aromatic hydrocarbons (PAHs), 22 metallic trace elements (ETM), 6 ions in addition to paraffins were identified on the collected PM<sub>2.5</sub>. The total PAHs, paraffins and ETM were respectively 4.88, 1062 and 10686 ng.m<sup>3</sup>. Identification sources indicate that anthropogenic activities, including road traffic and the burning of biomass such as charcoal, are the major contributors.

#### **7.04.P-Mo429 Chemical Characterization And In Vitro Toxicological Effects of Atmospheric Pollution (PM<sub>2.5</sub>) Collected in Cotonou, Benin**

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Airborne particulate matter (PM), a major component of outdoor air pollution was also classified as carcinogenic. Moreover, the International Agency for Research on Cancer showed an increasing risk of lung cancer with increasing levels of exposure to PM. Hence, it is somewhat critical to have a better knowledge on the air pollution PM in Africa. After particulate matter (PM)

sampling in Cotonou (Benin), a complete physicochemical characterization of PM<sub>2.5</sub> and PM<sub>>2.5</sub> was led. Average concentrations of air particulate matter were 180.9 µg/m<sup>3</sup> and 94.5 µg/m<sup>3</sup> in PM<sub>2.5</sub> and PM<sub>>2.5</sub>, respectively. Total PM number with aerodynamic diameter ≤ 2.5 µm was 97.5 % in both PM. The most abundant water-soluble ions concentrations recorded were Ca<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, Na<sup>+</sup> and Cl<sup>-</sup> for both PM. Moreover, chemicals concentrations were almost two fold higher in PM<sub>2.5</sub> than in PM<sub>>2.5</sub> with total metals of 10.78 µg/m<sup>3</sup> found in PM<sub>2.5</sub> versus 5.68 µg/m<sup>3</sup> in PM<sub>>2.5</sub>. Identification tools of major pollution source as inorganic elements, paraffins, fatty acids ratios and PAHs ratios indicated that PM under study were originated from traffic exhaust. Then, their adverse health effects were evaluated by using *in vitro* culture of human lung cells. BEAS-2B cells (bronchial epithelial cells) were intoxicated during short-term exposure at increasing PM concentrations (1.5 to 96 mg/cm<sup>2</sup>) to determine global cytotoxicity. Hence, cells were exposed to 3 and 12 mg/cm<sup>2</sup> to investigate the potential biological imbalance generated by PM toxicity. Our findings showed the ability of both PM to induce oxidative stress and to cause inflammatory cytokines/chemokines gene expression and secretion. Furthermore, PM was able to induce gene expression of enzymes involved in the xenobiotic metabolism pathway. Strong correlations between gene expression of metabolizing enzymes, proinflammatory responses and cell cycle alteration were found, as well as between proinflammatory responses and cell viability. Stress oxidant parameters were highly correlated with expression and protein secretion of inflammatory mediators.

## **7.05 Multi-Purpose Use of Wastewater Based Epidemiology for Assessing and Evaluating Public Health Status: Challenges and Opportunities on a Global Scale**

### **7.05.P-Tu433 Wastewater-Based Epidemiology As A High-Throughput Method To Investigate Human Exposure To Bisphenols. Method Development, Pilot Application and Contrast to Urinalysis**

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Bisphenols are widely used in plastic production and as additives. Thus, bisphenol A (BPA) and its most prominent substitutes, bisphenol F (BPF) and bisphenol S (BPS) have been detected in many environmental samples. Human exposure to bisphenols can be estimated by measuring the concentration of precursor and metabolites in urine. Alternatively, wastewater-based epidemiology (WBE) is a very promising methodology that can complement human biomonitoring.

In this context, the aim of this study was to develop an analytical methodology for the determination of 4 metabolites from BPA: monosulfate (BPA-S), disulfate (BPA-DS), β-D-glucuronide (BPA-G) and bis-(β-D-glucuronide) (BPA-DG), 2 metabolites from BPS: monosulfate (BPS-S) and β-D-glucuronide (BPS-G) and 2 metabolites from BPF: monosulfate (BPF-S) and β-D-glucuronide (BPF-G) in wastewater and to assess their usability as biomarkers for WBE. The method was applied to the analysis of wastewater samples from 4 Spanish cities (A-D) and 1 Portuguese city (E). Exposure to bisphenols at city C was also investigated by urinalysis as a pilot assay. The methods, based on solid-phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), showed quantification limits below 35 ng/L for metabolites in wastewater, and 2.2 ng/mL for bisphenols in urine

BPA-S, BPS-S and BPF-S were positively detected in wastewater samples. None of the compounds was detected in city B. The highest exposure levels were determined for BPA in City A and D (mean: 86-1231 µg/(day/1000inh.)) and for BPF in City A (206 µg/(day/1000inh.)). Human exposure to BPS in wastewater was also detected but at lower levels (41-100 µg/(day/1000inh.)). WBE-derived exposure was referred to average-body weight and compared with the tolerable daily intake (TDI) level for BPA, exceeding this threshold in Cities A and D. BPA, BPF and BPS were positively detected in the urine samples from City C. BPF was detected at the highest concentration and frequency, in 91% of the urine samples (median: 14.9 ng/mL), followed by BPA at a lower frequency (16%, median <1.1 ng/mL). BPS was also detected with high frequency but in general low concentration levels (82%, median: 0.90 ng/mL).

### **7.05.P-Tu434 Turning Wastewater Treatment Plants into Sentinels of Public Health: The case study of Larnaca**

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The COVID-19 pandemic has brought about globally concerted efforts in wastewater-based epidemiology (WBE), which have successfully focused on the detection of SARS-CoV-2 virus RNA in sewage wastewater, in order to better understand the dynamics and spatiotemporal trends of the pandemic.

An integrated, multi-disciplinary sewage-based surveillance system was implemented in Cyprus to track SARS-CoV-2 trends through various SARS-CoV-2 genetic marker concentration normalization approaches based on wastewater physicochemical characteristics, accompanied by epidemiological analyses. This system monitors all seven (7) urban areas and wastewater treatment plants (UWTPs) of Cyprus, in a national country-wide surveillance campaign for SARS-CoV-2 genetic markers during the pandemic. Herein, the case study of Larnaca is shown, whose influent urban wastewater was surveilled for a continuous period of 18 months (March 2021-September 2022).

Weekly SARS-CoV-2 genetic marker concentrations were normalized according to different physicochemical parameters (COD, TN, TP and NH<sub>4</sub>-N). The PE at any given time of sampling, were assessed for the best approximation to the UWTP-served population of 76000 PE, as provided by the Sewerage Board of Larnaca.

The most suitable physicochemical parameters for PE estimation were COD and TP. These gave the closest approximation to the UWTP-served population of 76000 PE. The highest N<sub>2</sub> concentrations were observed in July 2021 and in March 2022, using all four physicochemical parameters for concentration normalization. These were in agreement with the numbers of diagnosed COVID-19 cases.



The log<sub>10</sub> wastewater concentrations of N2 and E genetic markers showed a trend that followed that of the 7-day and 14-day log<sub>10</sub> cumulative number of cases. This fact shows the ability of the wastewater-based system to reflect the trends of COVID-19 cases in the UWTP-served community of Larnaca.

According to the findings of this study, COD and TP were shown to be suitable parameters for SARS-CoV-2 genetic marker concentration normalization in urban wastewater. This work also includes the wastewater-based surveillance of all 7 urban areas and UWTPs of Cyprus. Wastewater SARS-CoV-2 genetic marker concentrations agreed, and reliably tracked the number of diagnosed COVID-19 cases and pandemic dynamics in Larnaca. Through this wastewater-based surveillance system, the value of urban wastewater as a sentinel of public health was proven.

#### **7.05.P-Tu435 Evaluation of Wastewater-based Epidemiology of COVID-19 Approaches in Singapore's 'Closed-system' Scenario: A Long-term Country-wide Assessment**

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With the COVID-19 pandemic the use of WBE to track diseases spread has rapidly evolved into a widely applied strategy worldwide. However, many of the current studies lack the necessary systematic approach and supporting quality of epidemiological data to fully evaluate the effectiveness and usefulness of such methods. Use of WBE in a very low disease prevalence setting and for long-term monitoring has yet to be validated and it is critical for its intended use as an early warning system. In this study, two monitoring scenarios were used: (i) city wide monitoring (population 5,700,000) and (ii) community/localized monitoring (population 24,000 to 240,000). Additional epidemiological data gathered include the number of active cases detected and the geospatial distribution of public places visited by the infected individuals while contagious. Prediction of active cases by WBE using multiple linear regression shows that a multiplexed qPCR approach with three gene targets has a significant advantage over single-gene monitoring approaches, with  $R^2 = 0.832$  (RMSE 0.053) for an analysis using N, ORF1ab and S genes ( $R^2 = 0.677$  to  $0.793$  for single gene strategies). A predicted disease prevalence of 0.001% (1 in 100,000) for a city-wide monitoring was estimated by the multiplexed RT-qPCR approach and was corroborated by epidemiological data evidence in three 'waves'. Localized monitoring setting shows an estimated detectable disease prevalence of ~0.002% (1 in 56,000) and is supported by the geospatial distribution of active cases and local population dynamics data. Data analysis also shows that this approach has an limitation in sensitivity, or hit rate, of 62.5 % and an associated high miss rate (false negative rate) of 37.5 % when compared to available epidemiological data. Nevertheless, our study shows that, with enough sampling resolution, WBE at a community level can achieve high precision and accuracies for case detection (96 % and 95 %, respectively) with low false omission rate (4.5 %).

#### **7.05.T-01 Use of a Freshwater Bivalve to Assess the Viral Risk of Water Bodies**

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Faecal contamination of freshwater is a source of diffuse pollution, which could carry pathogenic microorganisms. Among them, enteric viruses could be responsible of waterborne outbreaks. Their direct detection in water is too expensive and difficult to set up on routine analyses. So, indicators of faecal pollution usually used, such as the bacteria *Escherichia coli*. Nevertheless such bacteria indicator is not enough reliable to estimate the viral hazard. Thus, many studies also suggest that F specific RNA bacteriophages could be used as indicators of enteric viruses in food and in water environments. F specific RNA bacteriophages (FRNAPH) display similar morphology than enteric viruses such as noroviruses and these infectious particles can be easily quantified in simple and complex matrices using standard or published methods. However, the detection of infectious FRNAPH in water has also several limits including the low loads in some situations, requiring the filtration of a large quantity of water, and the variability of the results due to the dynamic character of the matrix (rivers).

To improve viral detection in freshwater environments, we propose to use a sentinel species (the freshwater bivalve mollusc *Dreissena polymorpha*) as a biosensor, due to its bioaccumulation capacities. This bivalve mollusc is already used as model in ecotoxicological studies to detect chemical such as persistent organic pollutants and microorganisms (i.e. bacteria, protozoan parasites). In this study, we aim to answer 3 questions: i) Do *D. polymorpha* accumulate FRNAPH in a dose-dependent manner? ii) Do FRNAPH concentrations in *D. polymorpha* remain in equilibrium with their environment contamination level during exposure? and iii) Do *D. polymorpha* keep the FRNAPH signal even after the exposure (depuration kinetics)?

The results show that *D. polymorpha* is able to accumulate infectious FRNAPH in laboratory conditions as demonstrated in a previously study, that is of a great interest for in situ application. Infectious FRNAPH concentrations in mussels reflect the exposure load in only 24 hours. The depuration phase seems to be exponential. The kinetics of accumulation and depuration of viral particles in mussels will be formalize through a toxico-kinetic model to describe the relationship between FRNAPH load in the mussels and the level of contamination of the water for monitoring purposes. *In situ* experiments will be performed by active transplantation to promote this outcome.

#### **7.05.T-02 Assessing the Exposure of Italian Population to Food Contaminants Through Wastewater-Based Epidemiology**

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Food safety is an issue of concern for public health, as foodborne illnesses are an important cause of morbidity and mortality worldwide, with a considerable impact also on socioeconomic development. Foodborne diseases are mainly caused by bacteria,

viruses and parasites, or by chemical substances entering the body through contaminated food and/or water. Food consumption is one of the most important routes of human exposure to contaminants, including natural (e.g. mycotoxins), process (e.g. acrylamide) or environmental pollutants (e.g. pesticides). Different indicators are normally combined to evaluate population exposure to food contaminants, such as human biomonitoring studies (HBM) and intake estimations. These approaches face several limitations and therefore, novel tools are needed to monitor rapidly and objectively human exposure to contaminants and support public health authorities.

This study aimed to (i) assess the capability of wastewater-based epidemiology (WBE) to evaluate community-wide exposure to food contaminants, especially mycotoxins, (ii) apply WBE approach in the first nationwide monitoring performed in Italy and (iii) evaluate spatial and temporal patterns of exposure. Urban wastewater samples were collected from 14 Italian cities during a 3-years period (2020-2022). Quantitative analysis of selected biomarkers was carried out by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry to achieve the low levels of these contaminants expected in wastewater. For mycotoxins, fumonisins and deoxynivalenol (DON) were found in almost all cities during the investigated period, being DON the most frequently detected in accordance with HBM studies. Mass loads were back-calculated and statistically compared, evidencing spatial and temporal patterns of exposure. Mass loads were also used for calculating daily intake and WBE results for mycotoxins were normally lower than the safety values proposed by different food organizations, demonstrating no risk for human health. WBE is a reliable complementary tool for evaluating community-wide exposure to food contaminants, able to enhance the information currently provided by HBM studies and intake estimations. Thus, WBE can be an additional indicator for public authorities to drive prevention strategies concerning food safety.

#### **7.05.T-03 Application of Wastewater-Based Epidemiology as a Tool to Assess Public Health – A Case Study in the UK during SARS-CoV-2 Pandemic**

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Non-communicable diseases (NCDs), also known as chronic diseases, are responsible of 41 million deaths/year, corresponding to the 74% of all yearly deaths. They are characterized by long duration, slow progression, non-transmissibility, and reduced quality of life. Current approaches to assess public health involve surveys, morbidity, deaths, prescriptions, and sales data. These tools are not feasible to obtain reliable pictures of health status as they contain many uncertainties (e.g. bias from self-report, incomplete treatment plan, different report formats). For this reason, a comprehensive approach is needed to provide accurate data to monitor health status of communities and reduce risks factors related to NCDs. Wastewater-based epidemiology (WBE) enables a systematic approach to estimate trends in consumption of pharmaceuticals and evaluate health status of population with high resolution. Human markers (e.g. pharmaceuticals) are excreted by individuals into urban sewer networks; as such, influent wastewater (IWW) can be considered as an invaluable source of information related to population's health; through the quantification of target compounds, it is possible to obtain information about pharmaceutical consumptions and lifestyle for a given wastewater catchment area. In this study, WBE was applied to assess public health status of communities in Southwest of England during Covid19 pandemic estimating pharmaceuticals consumption. Two 24h-composite IWW samples/week were collected for two years (Mar20-Mar22), processed using solid-phase-extraction and analysed via UPLC-MSMS. Daily loads (DLs) were calculated, considering concentrations, wastewater flows, and metabolism correction factors. Normalization of DLs using population served per catchment allowed comparisons among different-sized communities and results were overlaid with Covid19 cases. Using this approach, it was possible to identify trends in pharmaceuticals consumption at different stages of the pandemic. Data triangulation highlighted changes in the consumption during periods of high Covid19 cases and lockdowns; moreover, this study shows that WBE can be used as near real-time monitoring tool for selected pharmaceuticals as proxy for population's health.

#### **7.05.T-04 Estimation of Alcohol Consumption in the Spanish Population through the Analysis of Ethyl Sulfate in Wastewater**

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Alcohol is one of the most consumed psychoactive substances throughout the world and it is very socially accepted and integrated into everyday life. According to the Global status report on alcohol and health, pure alcohol consumption per person aged 15 or older in Spain was 10.7 liters per year (WHO, 2019). In this study, an alternative and complementary method to those approaches currently used to estimate alcohol consumption by the population is described. This method, wastewater-based epidemiology (WBE), allows back-calculating the alcohol consumption rate in each population from the concentrations of a selected biomarker measured in sewage waters.

A total of 28 wastewater treatment plants (WWTPs) were sampled in 22 Spanish cities that belong to 10 autonomous communities. Time-average 24h composite raw wastewater samples were collected from the studied WWTPs on consecutive days along 1 week in the spring and autumn seasons of 2021 and 2022. The EtS was analyzed using an analytical method based on ion-pair liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The alcohol consumption estimated from levels of EtS in the analyzed samples ranged from 4.5 and 46 mL/day/inhabitant, with significant differences between cities from different regions and between regions. Also, it is observed that consumption on weekends is higher than on weekdays, with average consumption rates more than 2.2 times higher from Friday to Sunday than from Monday to Thursday. The alcohol consumption investigated in 2021 and 2022 in comparison with previously reported data (in the Spring of 2018) shows the highest consumption values in 2018, the lowest in 2021 (when the COVID-19 pandemic restrictions were still important), and a recovery to intermediate values in 2022. WBE-derived estimates of alcohol consumption were comparable to previous studies and the values reported by the Spanish Ministry of Health, Consumption and Social Welfare in collaboration with the National Institute of Statistics (INE).

In conclusion, the study finds that the WBE approach is a very useful tool, complementary to traditional methods, to estimate the consumption of alcohol in a population.

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#### **7.05.P Multi-Purpose Use of Wastewater Based Epidemiology for Assessing and Evaluating Public Health Status: Challenges and Opportunities on a Global Scale**

##### **7.05.P-Tu436 Profiling of PAHs Biomarkers in Wastewater in the Aspect of Public Health Risk Assessment of Hazardous Chemicals Exposure at Urban Areas**

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There is strong evidence of health risks from human exposure to organic air contaminants such as PAHs (polycyclic aromatic hydrocarbons) from combustion processes (solid fuels, coal and biomass), as well as communication. Most of the high-molecular-weight PAHs are only slowly degraded and hence represent a long-term potential health hazard to living beings. There is a lack of knowledge and understanding of community-wide exposure to organic air pollutants, especially in highly urbanized areas in Eastern Europe.

PAHs entering the body in an some part are removed in unchanged form but mostly undergo few step process of biotransformation and detoxification process and then are excreted via urine and feces as their hydroxy metabolites.

The aim of the study was the determination of the hydroxyl PAH metabolites profile in wastewater. The stability of three OH-PAHs in wastewater was confirmed in the first step of our studies and was presented in J. Durak et al. (J. Durak et al. 2021).

The 24 composite samples of wastewater influent were collected at the “Plaszow” wastewater treatment plant (WWTP) in Krakow, in winter and summer. This plant is the biggest one in the city and the third in the country, treating over a 70% of Krakow wastewater from over 480 thousands of inhabitants in the central part of the city, with an average capacity of 165.000 m<sup>3</sup>/day.

Studies were carried out using SPE-GC-MS/MS method for the determination of selected OH-PAH (1-hydroxy and 2-hydroxynaphthalene (1-OH-NAP, 2-OH-NAP), 2-hydroxy and 9-hydroxyfluorene (2-OH-FLU, 9-OH-FLU), 9-hydroxyphenanthrene (9-OH-PHEN), 1-hydroxypyrene (1-OH-PYR), 3-hydroxybenzo(a)pyrene (3-OH-BaP)) in samples collected in WWTP. Analysis was carried out by means of a Thermo Scientific GC TRACE 1300 (GC-(IT)MS/MS) and a TriPlus RSH Autosampler.

The average concentrations of compounds ranged from 5 ng/L to more than 400 ng/L. The highest concentrations of OH-PAH were detected in the influents, for 2-OH-NAP and 9-OH-FLU in winter and summer. However, their concentrations were twice lower in summer. A similar trend was observed for other compounds. 1-OH-PYR was observed for influent and effluent samples only in winter at average concentrations of 8 ng/L and 5 ng/L, respectively.

##### **7.05.P-Tu437 A Novel Analytical Tool Using HRMS (Orbitrap) for the Estimation of Drugs of Abuse Consumption through Wastewater-based Epidemiology: Development & Application in Real Samples from Valencia, Spain**

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Wastewater-based epidemiology (WBE) estimates the consumption of drugs of abuse in any given population. Developing methods for the rapid screening and confirmation of drugs of abuse and their metabolites is still a priority. A method to quantify six illicit substances indicators using solid-phase extraction (SPE) and UHPLC-HRMS/MS (Orbitrap Exploris 120) was developed, validated and applied to the analysis of these wastewater samples. As far as the HRMS/MS system is concerned, the correct tuning of instrumental parameters was crucial to achieve selectivity, sensitivity and reproducibility for the analytes.

Different acquisition modes are available while using a Q-Orbitrap platform and the optimized protocol focused on targeted analysis. The best results were obtained by applying a full scan (FS) and, then, a target MS<sup>2</sup> (ddMS<sup>2</sup>) using an inclusion list that contains the most important drugs and a filter for dynamic exclusion. Amphetamine, benzoylecgonine, cocaine, methamphetamine and ecstasy were detected in positive mode, while 11-Nor-9-carboxi-delta-9-tetrahydrocannabinol, the main secondary metabolite of cannabis formed in the human body after consumption, was detected in negative mode. Intra- day precision (% RSD,  $n = 3$ ) ranged from 0.03 to 18.7 %, while linearity ( $R^2$ ) values were higher than 0.9970 and the lowest method detection limit was 0.01 µg/ ml. The method was applied for the estimation of drug consumption in wastewater samples taken from the two main wastewater treatment plants of Valencia city during a whole week in November. Cannabis and cocaine estimated consumptions remain the highest in Valencia during the last 10 years, with calculated amounts up to 66 g/day/1000inh and 9 g/day/1000inh, respectively. Higher consumption during weekends was observed, mainly for recreational reasons.

#### **7.05.P-Tu438 Wastewater-Based Epidemiology for Monitoring the Evolution of SARS-CoV-2 in Different Spanish Municipalities**

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Analysis of urban wastewater has shown great potential for monitoring the evolution of COVID-19 pandemic within communities. It is a fast and cost-effective tool that enables tracing of both symptomatic and asymptomatic transmission of SARS-CoV-2. Measuring the SARS-CoV-2 RNA in wastewater has been of help to evaluate the evolution of the pandemic and to take appropriate measures to face this problem. However, some critical issues related to the type of samples collected, the stability of the RNA material, and back calculation aspects including estimation of the number of inhabitants and mobility of the population, have been less studied.

In this work, we have applied the general WBE strategy to a wide study performed in several municipalities from the Castellon province, Spain. The above-mentioned critical issues are discussed, together with the stability of samples under different storage conditions. Special attention is drawn to the comparison of 4h or 24h-composite samples to better monitor SARS-CoV-2 in wastewater. The evolution of the pandemic in the first steps of 2020 is illustrated with data collected from RTq-PCR analysis, and data provided by wastewater analysis are interpreted and compared with a wide set of epidemiological indicators (the day of onset of symptoms, date of diagnosis, date of declaration, date of admission to hospital care) for validation of the approach used. This is a comprehensive study in which a combined analysis of epidemiological data as well as WBE-driven data at the same municipalities are compared and discussed. may help other researchers to standardise or improve their sampling and storage protocols for SARS-CoV-2 detection and quantification in wastewater to gain confidence in the interpretation of the results.

#### **7.05.P-Tu439 Wastewater, Useless but Valuable – A Scientific Perspective**

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Wastewater is a reservoir for measuring infection levels, illicit drugs or chemicals in a given community. The importance of wastewater became more popular recently because of the SARS-COV-2 surveillance. Future pandemic surveillance can be done if the knowledge gained from SARS-CoV-2 RNA monitoring in wastewater can be preserved and improved. This work will focus on the advantage of monitoring SARS-CoV-2 RNA from wastewater, the lesson learned during the COVID-19 pandemic, and the problem to avoid in the future. Wastewater surveillance data from Saskatchewan, Canada, was a critical tool for decision-making during the Omicron wave when clinical data became unavailable. One of the advantages of wastewater data is that it can be made public without compromising anyone's right to privacy. Wastewater surveillance gave an early warning for the next wave for all waves since 2021, which, if capitalized on, could help with proper planning. New variants of concern (VOC) were also detected in wastewater before evidence in clinical samples. Most importantly, all circulating variants can be captured through wastewater whole genome sequencing, which might not be possible in clinical samples. Effective reproduction number has also been developed from wastewater data, which is helping to understand the surveillance data. Other models which are helping to predict the communal cases and hospitalization data have been generated from wastewater surveillance. The recent surveillance work has shown the importance of collaboration between health authorities, government agencies and researchers. The unavailability of clinical data limited the extent to which models can be developed to aid future research work in most cities.

#### **7.05.P-Tu440 Drug Consumption in German Cities and Municipalities During the COVID-19 Lockdown: A Wastewater Analysis**

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Analysis of illicit drugs, medicines and pathogens in wastewater is a powerful tool for epidemiological studies to monitor public health trends. Since the spread of the coronavirus disease (COVID-19) worldwide, wastewater-based epidemiology (WBE) has become an additional tool to monitor the prevalence of SARS-CoV-2 infections at local or even national level. The samples collected for this purpose can also be used for numerous other epidemiological studies.

The aims of this study were to (i) assess spatial and temporal trends of population-normalized mass loads of illicit drugs and nicotine in raw wastewater in the time of regulations against SARS-CoV-2 infections (2020–21); (ii) find substances that are feasible markers for characterizing the occurrence of selected drugs in wastewater.

Raw sewage 24-hour composite samples were collected in catchment areas of 15 wastewater treatment plants (WWTPs) in urban, small-town and rural areas in Germany during different lockdown phases from April 2020 to December 2021. Parent substances (amphetamines, methamphetamine, MDMA, carbamazepine, gabapentin and metoprolol) and the metabolites of cocaine (benzoylecgonine) and nicotine (cotinine) were measured.

The daily discharge of WWTP influents were used to calculate the daily load (mg/day) normalized by population equivalents (PE) in drained catchment areas (in mg/1,000 persons/day). An influence of the regulations to reduce SARS-CoV-2 infections such as contact bans and border closures on drug consumption has been proven in some cases and refuted in several. In addition, metoprolol and cotinine were found to be suitable as marker substances for the characterization of wastewater. A change in drug use was visible at the beginning of the Corona crisis. Thereafter from mid-2020, no obvious effect was detected with regard to the regulations against SARS-CoV-2 infections on concentration of drugs in wastewater. Wastewater-based epidemiology is suitable for showing changes in drug use during the COVID-19 lockdown.

#### **7.05.P-Tu441 Neurodegenerative Disease Prevalence Rates and Sewage Sludge Heavy Metals in the United States (2017-2022)**

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Neurodegenerative diseases (NDDs) such as Alzheimer's Disease (AD), Parkinson's Disease (PD), and amyotrophic lateral sclerosis (ALS) require extensive testing over months or years before a diagnosis is established and treatment can begin to improve survivorship and quality of life. Studies indicate strong associations between environmental exposure to heavy metals (e.g., cadmium, arsenic, manganese, and mercury) and NDDs. Varied regional NDD prevalence rates throughout the United States suggest that certain environmental conditions (e.g., contamination from industrial runoff) could increase exposure to heavy metals in certain populations, thereby increasing the risk of NDD development. Municipal sewage sludge reports of heavy metal concentrations regulated by the Environmental Protection Agency could serve as viable data sources to assess the relationship between environmental exposure and NDD prevalence rates. This study aimed to determine whether heavy metal concentrations correlate with NDD prevalence rates. We hypothesize that heavy metal concentrations in sewage sludge positively correlate with regional NDD prevalence rates (95% CI). Sewage sludge reports from wastewater treatment plants in the Human Health Observatory network were analyzed for the maximum and monthly average concentrations of arsenic (75 and 41 mg/kg, respectively), cadmium (85 and 39 mg/kg), and mercury (57 and 17 mg/kg). The low observable adverse effect level (LOAEL) was used as the benchmark to distinguish between high and low concentrations for arsenic and mercury, whereas the no observable adverse effect level (NOAEL) was used for cadmium due to limited human data. Furthermore, average individual exposure was calculated using census population density estimates. Expected results are that sites with cadmium concentrations above the NOAEL (0.005 mg/kg/day), arsenic concentrations above the LOAEL (0.014 mg/kg/day), or mercury concentrations above the LOAEL (0.003 mg/kg/day) will also report prevalence rates (per 100,000 people) above the mean for AD (184.0), PD (245.7), and ALS (4.9). Identifying NDD-susceptible populations prior to symptom onset has the potential to reduce diagnostic delays and extend or improve quality of life. Importantly, if this research finds a positive correlation between heavy metal concentration and NDD prevalence, it has the potential to raise awareness of this risk factor and encourage screenings for NDDs earlier in life for susceptible populations.

#### **7.05.P-Tu443 Wastewater based Surveillance: More Than a One Trick Pony**

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Following the identification and spread of SARS-CoV-2, scientists worldwide and especially the international water community rapidly developed methods to quantify the viral genetic signal in untreated water. Wastewater based epidemiology (WBE), and methods associated with this field now act as complementary tools to clinical testing for SARS-CoV-2, or as a replacement to clinical testing whereby individual testing has become less common over time. To date, wastewater-based surveillance has been primarily used to evaluate SARS-CoV-2 spread but has recently begun to expand its domain of usage to include infection trends of gastrointestinal diseases, but also respiratory diseases such as respiratory syncytial virus (RSV). Here we report that monkeypox virus can be detected in wastewater composite supernatant-solid in two wastewater facilities, but also at various institutions including correctional, assisted living and university dormitories. Unsurprisingly, certain locations demonstrated higher viral loads than others. In general, monkeypox testing is limited in these locations, making it difficult to determine the strength of the association between viral quantification in water and positivity rates at the community level, with wastewater testing the only means to monitor community spread currently. As such, WBE represents an important tool to augment monkey pox and other virus surveillance and public health response efforts.

#### **7.05.P-Tu444 Variation in Locational Response to Omicron, The Emergence of Cryptic Lineages?**

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In late 2021, the variant named Omicron was identified due to an S gene drop out during regular sample checking in South Africa. This variant spread extraordinary rapidly with the initial wave of BA.1 from December to February 2022, followed by the emergence of another subvariant named BA.2. Regular monitoring of wastewater treatment plants under an ongoing wastewater-based surveillance study with the state of Texas identified Omicron two weeks prior in Denton before its detection in Waco. hereafter, it was detected in numerous other facilities. Sampling consisted of (a) once weekly sampling and analysis of wildtype (N1, N2) and omicron specific indicator (P13L), (2) normalization to fecal indicator (PMMoV) and (c) comparison with active cases/hospitalizations. The time profiles of SARS-CoV-2 in wastewater broadly matched the waves of active cases, but with two notable differences between assisted living facilities (n = 5) and correctional facilities (n=6). For these two locations, differences were detected in the frequency of positive detects for targets, with assisted living facilities having less than 50 % agreement between targets compared to > 90 % agreement between targets for the correctional facilities (> 100 % agreement up to April). Samples have been sent for sequencing to determine if the assisted living facilities represent a potential source for the emergence of mutations/cryptic lineages given the suggested frequency of drop offs in nucleotides suggested by the results, the results of which will assist in improving our understanding of the spread and mutations associated with this ongoing infectious disease.

## **7.06 Next Generation of Risk Assessment and Management of Chemicals to Address Nature Preservation and Ecosystem Services**

### **7.06.T-01 Conflicting Priorities in the Environmental Risk Management of Plant Protection Products (PPP)**

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In the European Union, a variety of European and national programs as well as strategies have been established in recent years with the aim to minimise the environmental exposure to chemical substances. As chemical formulations being directly emitted into the environment, plant protection products (PPP) are addressed in several of these strategies. One prominent example is the European Green Deal with its Farm-to-Fork strategy for a fair, healthy and environmentally-friendly food system. In this strategy, targets have been defined for an union-wide extensive reduction of chemical PPP use and a substitution by less hazardous substances, an increasing use of non-chemical alternatives, an enforcement of integrated pest management, an increase in organic farming and reduction or ban of chemical PPP in sensitive areas, among others.

For a sustainable design of plant protection, a multi-dimensional analysis of the environmental and socioeconomic impacts of each measure is required. For instance, the effects of herbicides on non-target organisms need to be assessed in comparison with the impact of ploughing on the natural soil habitat, enhanced erosion and an increase in the CO<sub>2</sub>-footprint due to longer driving times. Another example is how the progressive use of digitalisation in farming on the one hand side contributes to a reduced and more precise PPP use. On the other hand, it stimulates the (re-)establishment of large-scale agricultural landscapes, counteracting the need for small-scale landscapes for preservation of ecotones. These analyses prevail the challenges of balancing different ecosystem services, in particular food security and biodiversity.

In addition to these measures, a paradigm shift in the PPP authorisation itself might be required. Today, an authorisation is in general granted on a national scale in conjunction with risk mitigation measures to be applied independent on the regional conditions. An authorisation (or rejection) of PPP for use in specific regional areas instead of an authorisation on an entire national scale might allow for a more precise risk management and at the same time pest control and resistance management. Challenges of such an approach are, among others, technical prerequisites, the communication of measures and restrictions to the end user and the possibility to control these specific mitigation instruments, for instance by robust monitoring programmes.

### **7.06.T-02 Availability and Applicability of Biodiversity Field Data to Enable Assessment of Biodiversity Loss Caused by Organic Pollution on a European Scale**

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The biodiversity of freshwater ecosystems is declining faster than that of any other ecosystem. The water resources are affected by multiple stressors as a result from drivers including urban and agricultural land use, hydropower generation and climate change. Actions to secure freshwater biodiversity have been inadequate and no global framework exists to guide policy responses with the urgency needed. A major challenge here is to develop methods for diagnosing the relative importance of organic pollution among other stressors present in freshwater ecosystems. In order to assess such broad-scale questions about freshwater biodiversity dynamics, it is urgent to assess freshwater diversity with proper biodiversity indicators. This may be hampered by data confidentiality, usage restrictions or limited accessibility, or data integration and quality issues despite universal acceptance of the FAIR (Findable, Accessible, Interoperable, Reuseable) data principles. The goal of this study is to establish the availability and applicability of biodiversity field data of European rivers and whether this data is suitable to enable assessment of biodiversity loss caused by pollution on a European scale. We will present available abundance data of geographical Europe. Presented data will be compiled, aligned and harmonized in terms of sampling protocols and taxonomical level. Biodiversity indicators will include species richness and species abundance. Discussion will be on the quality and the quantity of the biodiversity indicators to estimate the applicability of an eco-epidemiology study on European scale.

### **7.06.T-03 Calibrating Predicted Ecotoxicity Effects to Observed Species Loss By Using Ecological Models**

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Linking ecotoxicity impacts to species loss is relevant for translating model-predicted species-level effects to damage on functional diversity and further to damage to ecosystem services. There are ~12,000 chemicals with available measured in vivo effect test data, enabling quantification of predicted impact of chemicals and mixtures. However, we still lack a refined calibration between predicted and observed impacts in terms of species loss. To address this gap, we introduce a systematic approach that consists of a stepwise procedure to plot raw data from all sampling sites, derive quantitative insights into toxic pressure as a limiting factor for species and biodiversity, and apply refined methods, such as Generalized Linear- and Generalized Additive Models and TITAN (Threshold Indicator Taxa Analysis), to finally yield insights about species loss. Our approach is used to derive data-driven extrapolation factors for linking the potentially affected fraction of species (PAF) for a given chemical to the potentially disappeared fraction of species (PDF) for use in life cycle impact assessment (LCIA) and chemical risk assessment. We start from the Netherlands big (bio-) monitoring data set; we first grouped the data into sub-regions with similar background characteristics, calculated mixture toxicity pressure (msPAF) at the level of chronic EC10 equivalents (msPAF-EC10eq), mapped macrofauna data and mixture toxicity pressure information based on spatial information and modeled the species abundance changes as functions of changes in the mixture toxicity pressure. In our results, we found taxa responding sensitively to low mixture exposures (i.e., showing abundance decline), while we also found some more tolerant taxa with abundance increasing up to moderate mixture exposures. Abundance and biodiversity generally decline upon exceedance of protective standards, and the data allow to calibrate PAF to PDF relationships. Ecological models, such as TITAN, can thus be used to determine consistent extrapolation factors to derive the effect-to-damage relationships required in different decision-support tools.

#### **7.06.T-04 iTrackDNA: Breaking Down Barriers for Confident Environmental DNA Adoption in Environmental Surveys and Decision-Making**

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The analysis of environmental DNA (eDNA) - genetic material shed from organisms into their environment - promises to provide rapid, non-destructive, accurate and cost-effective biodiversity information. However, inconsistent practices and poor quality eDNA detection tools threaten End-User (regulators, industry, Indigenous Peoples, NGOs) uptake because of unacceptably high false negatives and false positives that can compromise effective management decision-making.

iTrackDNA is a multi-year, large scale applied research project that is addressing these concerns with researchers and End-Users across Canada and sectors. It is building End-User capacity through innovative, accessible, socially responsible genomics-based analytical eDNA tools for effective decision-making by: 1) supporting the creation of a targeted eDNA detection national standard; 2) building eDNA kits to detect 100 priority invertebrates, fish, amphibians, birds, reptiles, and mammals in Canadian coastal and inland ecosystems; 3) applying 10 eRNA kits for determining animal biosurveillance, biosanitation, and bioremediation effectiveness; 4) generating decision support software for modeling regional biodiversity changes integrating Indigenous Ecological Knowledge; 5) developing an eDNA training, certification, and inter-lab validation framework for consultants, researchers, regulators, and managers; and 6) producing a guidance document on eDNA-based methods integration into management, policy & regulations.

Recent progress will be presented to highlight activities to build and augment the eDNA community of practice through national eDNA standards adoption and transformative testing to confidently enable eDNA applications in coastal and inland ecological surveys and biosurveillance for mining, forestry, energy, and infrastructure projects.

#### **7.06.T-05 Simulating The Effects of Chemical Stressors on a Recreational Fishery: An Ecosystem Services Approach to Risk Assessment**

**Susanna Mõlkänen**, Lorraine Maltby and Philip Warren, University of Sheffield, United Kingdom

Recreational fisheries are a cultural ecosystem service providing recreation for millions of anglers globally. In freshwaters, such fisheries are under increasing pressure from chemical stressors and provide a good example of an environment where the ecosystem service approach can be applied to chemical risk assessment. By modifying the ecological model InSTREAM v7.3, an individual-based model (IBM) on salmonids, we can simulate the effects of chemical stress on a recreational fishery to determine the response of the angling stock. InSTREAM v7.3 was modified to include the effects of chemical stressors on chemical effect endpoints in trout individuals, including growth, reproduction, and survival as well as on an indirect endpoint, prey density. These endpoints were tested under varying exposure scenarios with and without recovery to determine the changes in the trout populations in a river reach. This was achieved by simulating varying levels of endpoint inhibition from no effect to full endpoint inhibition. The changes in the trout population were then equated to angling stock (trout older than 1 year and larger than 20 cm in length). The angling stock in the recreational fishery is then equated to ecosystem service delivery through angler satisfaction. For the chemical effect endpoint of growth rate, the angling stock stabilises in continuous exposure scenarios, however, it decreases by up to 83% of the baseline angling stock until thirty percent of growth rate where the angling stock is fully depleted. When the trout population is simulated with a recovery period following five years of chemical exposure, the angling stock recovers to baseline conditions in all levels of growth rate. The results vary for the different endpoints and will be discussed further. This study demonstrates the use of an IBM approach for linking chemical exposure effects on individual organisms to ecosystem service outcomes. Moving forward the combined chemical effects will be investigated as well as identifying the influence of spatial scale on the ability of recreational fisheries to tolerate chemical stress. This shows how adapting an ecological model can be used to address ecosystem service delivery in chemical risk assessment for a recreational fishery.

## 7.06.P Next Generation of Risk Assessment and Management of Chemicals to Address Nature Preservation and Ecosystem Services

### 7.06.P-Tu445 Risk Assessment to Support and Promote Efficient Overall Protection of Biodiversity – An Activity Within the Horizon Europe Partnership for the Assessment of Risk from Chemicals

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As a response to the EU Green deal initiatives Farm to fork, Biodiversity, Soil and Chemicals strategies an activity has been set up within the 7-year Horizon Europe Partnership for the Assessment of Risk from Chemicals (PARC) to improve the environmental risk assessment (ERA) of plant protection products (PPP).

This activity will explore a holistic scientific reframing of the regulatory ERA to more realistically assess ecosystem impact of plant protection products (PPPs) and to overcome the shortcomings of the current substance-by-substance assessment paradigm. The activity will investigate possibilities to simplify and focus the methods used for the ERA of PPPs.

The activity involves 20 European research partners covering competences in ecotoxicology, chemistry, ecology, environmental monitoring, systems science, environmental modelling and computer science. The work is divided into four main projects, Exposure, Effects, Benchmark and Landscape ERA. Projects Exposure and Effects aim to consolidate and update prospective ERA models by strengthening the feed-back loop from environmental monitoring and by doing so investigate possibilities to reduce complexity in the assessments. Monitoring data on exposure and effects of PPPs from different regions in Europe are assembled. Prospective ERA methods of varying degree of detail will also be tested in parallel. The results from comparisons of prospective ERA methods versus monitoring data will feed into other projects Benchmark and Landscape ERA aiming to 1. improve comparability between assessments for different PPPs, and 2. develop holistic approaches to assess effects of multiple PPP use and ecosystems stressors at the landscape level.

A key objective of the PARC research is to warrant regulatory feasibility and implementation of new concepts and tools. To this end a managing subtask engaging regulators and other stakeholders has been set up to ensure regulatory relevance and feasible time perspectives for regulatory implementation. The PARC activity aims at facilitating the transition towards a next generation, systems-based ERA.

### 7.06.P-Tu446 Science-Based Recommendations to Address Chemical Pollution as a Threat to Biodiversity

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Chemical pollution is a threat to biodiversity worldwide. This has been recognized in the Post-2020 Biodiversity Framework, which proposes to regulate the release of chemicals into the environment and identifies specific indicators focusing on pesticides, nutrients and plastic waste (Target 7). We support the inclusion of these substances but believe that other groups of pollutants that may contribute to biodiversity loss should also be considered and included in biodiversity-related targets for the reduction of chemical pollution. Specifically, we propose to include per- and polyfluoroalkyl substances (PFAS), endocrine-disrupting chemicals (EDCs), non-agricultural biocides, and toxic metal(loid)s. In addition, data on emerging pollutants (e.g., industrial chemicals, liquid crystal monomers, pharmaceuticals, and personal care products) need to be regularly reviewed and added to Target 7 of the Post-2020 Biodiversity Framework in case of threats to biodiversity.

To protect ecosystems and biodiversity from those pollutant groups, various strategies exist to reduce emissions of hazardous chemicals through simplifying and grouping chemicals, reducing the production of chemicals that are not strictly needed and innovative synthesis strategies ("benign by design"). In this context, the entire life cycle of chemicals, i.e. production, use phase and end-of-life, must be considered and insights from different expert communities need to be bridged and translated to enable true interdisciplinary biodiversity-related risk assessments and consequential measures. As a crucial step in this regard, we propose to establish data inventories that provide transparent information on the production, transport and emissions of chemicals in cooperation with industry. These to date often hardly accessible data could serve as the basis for indicators to monitor the effectiveness of the pollution-related targets set post-2020 biodiversity framework and related activities.

### 7.06.P-Tu447 Building an European Partnership for Next- Generation Systems-based Environmental Risk Assessment (PERA) – A Project Developing a Roadmap For action

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The European Partnership for next generation, systems-based Environmental Risk Assessment (PERA) is a strategic initiative launched by the European Food Safety Authority (EFSA) in response to the recognition that the current ERA paradigm has “fallen out of step with scientific knowledge and progress” and the new policy and societal ambitions e.g. the EU Green Deal strategy to better safeguard the environment. EFSA commissioned the development of a roadmap to facilitate a transition to an integrated environmental risk assessment (ERA) approach. The PERA systems-based approach aims to connect relevant partners from various sectors across regulatory silos, promotes and facilitates the sharing of data and expertise, and improves efficiency and transparency of the risk assessment process. The PERA roadmap comprises a problem description (current state), a vision (future state) and a series of steps to move towards the vision (transition process). It was developed after extensive desk research and multiple stakeholder interviews, that were used to identify partners, areas needing further development, challenges, blockers and collaboration opportunities. We designed a vision for next generation ERAs, initially focused on pesticides, based on the ideas of stakeholders discussed during workshops and accounting for the identified strengths and weaknesses of the current ERA system. The PERA vision is a framework for systems-based ERA, with a concentric circle model. Its core circle builds upon and strengthens current ERA processes by focusing on pesticide specific aspects useful for comparability and decreasing the assessment complexity of pesticides. The inner core circle is surrounded by a supportive circle of landscape scale ERA, that addresses ecosystems properties of generic relevance for all pesticides. These two ERA circles are in turn connected to surrounding circles representing the agricultural, ecological and production systems as well as the political, economic and social systems. To fully optimize the process these circles need to be interconnected with information feed-back loops. The vision and roadmap were designed to incorporate the strengths, knowledge and expertise of the current state into a new holistically framed systems-based ERA. Strategies for transition, as well as concrete projects to spark the transition process are proposed and include concrete and tangible steps in a holistic direction within the current regulatory framework.

#### **7.06.P-Tu448 Predicting Pesticide Effects on Aquatic Community Endpoints in Rice Fields of Southern Europe – A Bayesian Network Approach**

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Bayesian network (BN) can aid some of the shortcomings of traditionally deterministic environmental risk assessment by better accounting and communicating uncertainty and variability. In our approach, they are used as a meta-model to predicting potential effect on different biological levels in the aquatic ecosystem. To achieve this, we incorporate inputs and outputs of a process-based exposure model (e.g., RICE Water Quality model - RICEWQ) and a probabilistic case-based effect model (e.g., Predicts the Ecological Risks of PESTicides - PERPEST) into BN models. The RICEWQ model was run with various scenario combinations based on meteorological, hydrological conditions, and agricultural scenarios. The PERPEST model predicts the effect on different biological endpoints using case-based reasoning applied to a database of microcosm and mesocosm experiments. In this study, we focused on the pesticide exposure in rice fields surrounding a Spanish Natural Park considering three selected pesticides: acetamiprid, MCPA, and azoxystrobin. The BN enabled the communication of uncertainties transparently connected to the predicted direct effect on exposure in the rice field and indirect effect on different biological endpoints, endpoint groups and community level for the aquatic environment. Also, with the developed model we can compare the different scenarios and pesticide types thereby aiding better informed decision-making.

#### **7.06.P-Tu449 Assessing In-field Effects of Pesticides in the Frame of the European Regulation and Implications for the Protection Of Biodiversity**

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The use of plant protection products (PPPs) plays a major role in the loss of overall biodiversity in agroecosystems [1,2]. The current legal framework stipulates that PPP “shall have no unacceptable effects on the environment...”, which is further specified by the consideration of the “*impact on non-target species*” and the “*impact on biodiversity and the ecosystem*” (Regulation (EU) 1107/2009). According to Reg. (EU) 283/2013 this includes also “*potential indirect effects via alteration of the food web*”. Nevertheless it is evident that PPPs have persistent negative effects on biodiversity [1].

In typical agricultural landscapes, a high percentage of the total area is covered by agricultural fields (“in-field areas”). Agricultural fields provide important habitats for non-target terrestrial plants (NTTPs) and arthropods (NTAs) that are highly dependent on in-field areas. These species are an integral part of the terrestrial biodiversity, and declining in-field populations can also affect the off-field area through altered meta-population dynamics. Nevertheless, in-field effects are not assessed at all for NTTPs and are likely severely underestimated for NTAs. Additionally, NTTPs and NTAs form the basis of the food webs, and population declines both in- and off-field can affect consumer species including farmland birds and mammals. The current environmental risk assessment of PPP largely ignores indirect effects via alteration of food webs [2]. Although these gaps are likely a major reason for the decline in biodiversity caused by PPPs, the protection requirements of Regulation 1107/2009 laid out above have still not been implemented 13 years after its entry into force.

To protect biodiversity and to satisfy legal protection requirements, the coming revision of the risk assessment guidance for NTPs and NTAs must include an appropriate assessment of in-field effects and indirect effects via alteration of the food webs. In our contribution we highlight current gaps and present a simple assessment scheme to broadly consider the outlined deficiencies in risk assessment until the respective guidance documents have been revised.

#### **7.06.P-Tu450 Towards an Ecosystem-based Approach to Environmental Risk Assessment for Freshwater Ecosystems**

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Ecological Risk Assessment (ERA) has been traditionally used in the environmental management of freshwater bodies to help evaluate the risk of anthropogenic pressures on the structure and function of ecosystems, including for data-poor scenarios. Conventional approaches to ERA predominantly evaluate the dose-response relationship of individual stressors attested on a limited range of taxa, which are used as proxies for predicting potential impacts on the freshwater ecosystem. ERA also addresses the associated uncertainty to account for the inter- and intra-species extrapolations, with the application of assessment factors, whose magnitude depends only on the availability and number of data points and test species. As a result, it does not directly consider all potential impacts of chemicals and their mixtures on the complex processes and interspecies interactions occurring in ecosystems. Yet, varying responses of different species to chemical exposure, and cascading ecological effects make outcomes difficult to predict at the community and ecosystem levels. Ecosystem-based management requires consideration of the whole suite of anthropogenic pressures affecting the entire freshwater ecosystem(s), rather than focusing on individual chemical and biological components. Recent advancements include understanding indirect effects, measuring functional compensation, assessing trade-offs, and including links to ecosystem services delivery, which are yet to be fully explored under an ERA context. Here, we review approaches currently available supporting ecosystem-based considerations and present a conceptual framework towards their integrated implementation, highlighting their role in operationalizing an ecosystem-based ERA for freshwater ecosystems. This framework builds on the existing methodologies for evaluating the cumulative impacts of multiple pressures on multiple ecosystem components and will propose a strategy towards making ecological risk assessment more representative of ecosystem-level responses.

#### **7.06.P-Tu451 Significant Improvement in Freshwater Invertebrate Biodiversity in English River Over The Past 30 Years**

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There remains a persistent concern that freshwater biodiversity is in decline and being threatened by chemical exposure. As the UK, and particularly England, is a densely populated nation with rivers of modest dilution capacity, this location is very suitable to examine how freshwater biodiversity has fared over the past 30 years. The biodiversity changing trends based on long-term dataset of 240,916 freshwater macroinvertebrates records from 1989 to 2018 for England was retrieved and examined from multiple characteristics (latitude, altitude, slope gradient and water flow). A sub-set of approximately 200 sites per English Region (1515 sites in total with 62514 samples), with the longest and most consistent records were matched with waste water exposure (predicted from the LF2000WQX model), upstream land cover (Land Cover Map 2015). To understand changes in aquatic macroinvertebrates diversity and sensitivity with respect to these parameters, the biotic indices of (i) overall community's family richness, (ii) Ephemeroptera, Plecoptera, Trichoptera (EPT) family richness, and (iii) the score used for reporting for the National River Authority (UK) and for the Water Framework Directive (EU) known as the Biological Monitoring Working Party (BMWP) were selected. It was found that for all altitude, latitude, slope gradient, river size, waste water exposure levels, and differing proportions of upstream woodland, semi-natural, arable and urban land cover, all biodiversity or sensitivity indices examined improved over this period, although this has slowed in some cases post 2003. On a range, the response of family richness against the tested multiple stressors has gone from 15 to 25 family groups, a 66% improvement over 30 years. The improvement in average EPT family richness (3 to 10 families, a more than three times gain), which are considered to be particularly sensitive to pollution, imply macroinvertebrates biodiversity is still benefiting from a national improvement in water quality. Separately, we found that whilst urban area in the catchment was a strong suppressor of biodiversity, those urban areas which contained a higher woodland percentage had a lower harmful effect. This provides support for Nature-based Solutions for the urban area.

#### **7.06.P-Tu452 Can Biofilm Functional Endpoints Reflect the Ecological Status of Rivers?**

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Rivers are one of the most impacted ecosystems worldwide, yet we need them to deliver a wide range of services for human populations. Within rivers, fluvial biofilms are especially connected to the capacity of the river to provide supporting environmental services, particularly nutrient and carbon cycling, but is it possible that those functional indicators reflect the ecological status of the river? To answer this question, we sampled epilithic and epipsammic (sediment) biofilms from 20 rivers along Catalonia (NE Spain): 7 classified as deficient/very deficient, 4 identified as mediocre and 9 classified as good/excellent ecological status by the Catalan Water Agency (ACA). We measured 3 functional aspects of biofilms: primary production, respiration and functional diversity by Community-Level Physiological Profiles using Biolog Ecoplates®. We hypothesise that good ecological status rivers will have higher functional diversity and a slightly positive net primary productivity (NPP), yet we seek to define these thresholds empirically.

Ecosystem functions are the basis of ecosystem services; therefore, the aim of this work is to characterise functional endpoints in fluvial biofilms, find patterns that help us distinguish between deficient and good status rivers and evaluate if they can be used in Ecological Risk Assessment (ERA) and as early warning signs of ecosystem service loss.

#### **7.06.P-Tu453 A Toolbox of Strategies to Allow Successful Reduction in Copper PPP Applications Aligned with the European Green Deal**

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Currently copper is approved as a fungicidal active substance for more than 50 different diseases in viticulture, horticulture, hops, market garden and arable crops. It is used, for example, in vineyards to control downy mildew (*Plasmopara viticola*), on arable crops to protect potato against late blight (*Phytophthora infestans*), in apple orchards to prevent the spread of scab (*Venturia inaequalis*), but also in many other crops. Although copper formulations have been used for more than 100 years, there are no reported resistances which makes it a very important tool in pest management systems for conventional farming and, particularly, for organic farming. In recent years regulatory restrictions on its use (a maximum number of doses per hectare and per year) have been implemented. Furthermore, the European Green Deal aims to reduce the use of chemical pesticides in agriculture by half by 2030 whilst also targeting an increase in organic production to 25% of agricultural land. These increased restrictions on the number of authorised applications of copper, and the ongoing threat of a total ban, present a challenge for growers, and particularly for organic growers, who are prohibited from using synthetic fungicides.

This study presents a critical review and summary of available scientific and technical information relating to reducing copper use whilst limiting plant health risk. A range of possible technical solutions were identified that include improved pesticide product formulation and application technology (including precision farming); better disease prediction tools and optimisation of application timing; and improved crop management processes to reduce disease. These potential solutions were assessed for their potential effectiveness in reducing pesticide input, cost and sustainability, and pathway to deployment, including how to incorporate these solutions into existing production/pest management systems and barriers to and conditions necessary for the adoption in the field. This toolbox aims to overcome producers' aversion to perceived risks (increased occurrence of disease) and provide them with a credible range of tools to reduce copper use while maintaining plant health and disease control. This will be essential to achieve the goals of the European Green Deal, and allow the sustainable use of copper pesticides to continue.

#### **7.06.P-Tu454 Testing a High Throughput Assay for DNA-based Monitoring and Assessment of Lake Quality in England**

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DNA analysis of biological samples for environmental assessment is undergoing rapid development and potentially offers a more cost-effective and efficient means of acquiring species data for ecological assessment, analogous to and broader than, that currently gathered by more established methods. Current statutory biomonitoring approaches are low throughput and focus on single taxonomic groups which can fail to capture wider community-level changes leading to shifts in ecological status. DNA based approaches can target multiple taxonomic groups, be analysed with a standardised pipeline to reduce bias inherent in visual identification of species and processed with high-throughput methods to increase data generation.

Here, we carry out a proof of concept study to test a DNA based assessment of 50 lakes across England. We use two sample types (water and biofilm) from lakes with a range of ecological quality scores, previously measured with standard methods. We use multi-marker metabarcoding to measure biodiversity from across the whole lake community in a high throughput manner.

We integrate community level biodiversity data and environmental data (water chemistry, climate data) using multi-view learning to identify signatures of lake quality and find the environmental factors driving changes in biodiversity in lakes of different qualities.

#### **7.06.P-Tu455 Assessing Risk of Offshore Windfarms Towards Ecosystem Services**

**Dries Fredericus Elisa Louis Lorré<sup>1</sup>**, Sara Vandamme<sup>1</sup>, Ulrike Braeckman<sup>1</sup>, Katrien Van der Biest<sup>2</sup>, Colin Janssen<sup>3</sup> and Jana Asselman<sup>4</sup>, (1)Ghent University, Belgium, (2)University of Antwerp, Belgium, (3)Laboratory for Environmental Toxicology (GhEnToxLab), Ghent University, Belgium, (4)Blue Growth Research Lab, Ghent University, Belgium

Offshore wind energy is widely regarded as one of the most credible sources for increasing renewable energy production. Currently, the Belgian offshore wind farms (OWFs) accommodate a total capacity of 2.26 GW and the installation of another 3.5 GW is proposed in the latest marine spatial plan. Although physical and biological impact of OWFs are frequently studied and become more and more understood, a comprehensive tool to evaluate changes in ecosystem services is lacking. Yet, all stakeholders could benefit from such a tool, as it enables intuitive evaluation of both environmental burdens and opportunities by monetizing the ecosystem services into "ecoservice capital". To this end, we propose a novel approach to assess the risks of OWFs on marine ecosystems. A set of ecosystem services were selected in collaboration with multiple key stakeholders. Ecosystem services were quantified using science-based models equations and driving parameters were selected. Environmental risk assessment (ERA) procedures were used to quantify the impact of OWFs on the ecosystem parameters, and subsequently coupled to the ecosystem services. This resulted in an evaluation of the impact and risk of changing ecoservice capital. As a first case study, waste remediation (as sedimentary nitrate reduction) was selected as ecosystem service. Total organic matter (TOM) and fine sediment fraction quantities were found to be good proxies within this model. As the Belgian part of the North Sea is one of the best studied and monitored marine areas in the world and OWFs are intensively monitored within this area, extensive data regarding these parameters is available and was used to construct predictive ERA models. Confirming previous results, no significant change in TOM was observed. However, the fine sediment fraction adjacent to turbines showed an increase of almost

5%. Based on these findings the impact is assumed to induce only low risk towards changing waste remediation potential of the environment, as the impacted area represents only 1.2% of the entire OWF area. This proof of principal study demonstrated the use of ecosystem service evaluations in past and present ecological risk assessment procedures. However, a more complete view and evaluation using other ecosystem services needs to be conducted to provide a more holistic view of sustainable design and developments of future offshore advancements.

## 7.06.V Next Generation of Risk Assessment and Management of Chemicals to Address Nature Preservation and Ecosystem Services

### 7.06.V-01 Artificially Mimicking Fluvial Ecosystems to Study the Influence of Biofilms on Toxicity Potential of Chemical Using Bioenergetics Traits of Fish (*Gambusia affinis*)

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Biofilms (BF) modulate the toxicity of chemicals by providing sites for biotransformation and/or chemical transfer to other organisms in fluvial ecosystems. Moreover, environmental variables influence the regulatory services of BF assemblages such as their capacity to oxygenate water and uptake the excess of dissolved nutrients. The effect on water quality due to reduced expression of these regulatory services can gravely affect the population fitness of higher trophic levels. An exposure study of 200µg/L of 1H-Benzotriazole (BTR) was simulated in the laboratory for 21 days by employing 12 microcosms (recirculating channels) with two functional groups: BF grown in artificial channels, fish kept in 90L aquaria and sediment in each aquaria to increase environmental realism. Four treatments used, with three replicates each, were: fish control (C), biofilm control with fish (BC), fish with BTR (FT) and biofilm, BTR and fish (BT). In the setup, water quality construed the BF functional performance. The study examined direct effects, without BF and indirect effects in the presence of BF, of BTR on bioenergetics traits of fish (standard metabolic rates SMR, maximum metabolic rates MMR and absolute aerobic scope AAS) as a proxy of population fitness. Interestingly, the fish controls (C) exhibited lower MMR ( $P=0.01$ ) and AAS ( $P=0.05$ ) than BF controls which demonstrates that there is an apparent sticky link between these two functional groups. BTR in presence of BF (BT) raised the maintenance cost (SMR) significantly ( $P=0.02$ ) compared to its control (BC) but the difference between C and FT; FT and BT; and BC and C remained insignificant ( $P=0.19$ ;  $0.66$  and  $0.12$ , respectively), demonstrating indirect effects. Similarly, the MMR, which determine the ability of fish to carry energetically expensive activities, was lower in the BT treatment compared to its control, BC ( $P=0.005$ ). The endpoint AAS carries functional value because it represents the energy available to carry simultaneous activities including growth and reproduction. We also found that BF influenced the toxicity by reducing AAS in BT treatments significantly ( $P=0.01$ ) relative to its control (BC). BF plays a significant role in defining the energy limits of fish and provides realism in the assessment of direct and indirect effects of chemicals on population fitness in fluvial ecosystems.

### 7.06.V-02 Effects of Palm Kernel Oil, Olive Oil, Crude Oil and Honey on Renal Function of Male Albino Rats

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This study investigated the effects of palm kernel oil, olive oil, crude oil and honey on renal function of male albino rats. These chemical substances are used in traditional medicine for various purposes, including as antidote for poisons. **Methods:** Thirty healthy male albino rats were purchased and used in this research study. The animals were randomly placed into five groups ( $n=6$ ). The animals were administered the corresponding chemical substances for a period of three weeks. They were later sacrificed and their blood samples and kidneys collected for biochemical and histological analysis respectively. **Results:** Urea increased in all the groups administered the different chemical substances compared to the control. The increase is statistically significant ( $p<0.05$ ) in groups 4 and 5, and non-significant ( $p>0.05$ ) in groups 2 and 3 when compared to the control (group 1). Creatinine increased non-significantly ( $p>0.05$ ) in all the test groups compared to the control. Sodium decreased non-significantly ( $p>0.05$ ) in group 2, but increased non-significantly ( $p>0.05$ ) in groups 3, 4 and 5 compared to the control. Potassium increased non-significantly ( $p>0.05$ ) in group 2, but increased significantly ( $p<0.05$ ) in groups 3, 4 and 5, while chloride increased significantly ( $p<0.05$ ) in groups 2, 3 and 5 and non-significantly ( $p>0.05$ ) in group 4 compared to the control. Photomicrographs of histoarchitectural state of the renal tissues showed some forms of alterations in some parts of the tissues of the test animals when compared with the control. **Conclusion:** This study showed that long term administration of palm kernel oil, olive oil, crude oil and honey, as used in this study could cause certain alteration to renal functions. The order of renal intoxication caused by the administration of the chemical substances is crude oil > honey > olive oil > palm kernel oil.

## 7.07 One Health: Ecotoxicology at the Human-Animal-Ecosystem Interface

### 7.07.T-01 One Health Approach for Evaluating Presence and Effect of Pharmaceuticals Discharged via a Wastewater Treatment Plant in the Marine Environment

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Marine sewage outfalls are major contributors of complex mixtures of pharmaceuticals, personal care products, other emerging contaminants and legacy contaminants, which together represent important ecological challenges in aquatic environments. Most traditional wastewater treatment plants are not designed to remove pharmaceuticals, which are increasingly used, especially after the pandemic. To face such challenge the One Health approach has been chose in order to combine the impact of discharged

pharmaceuticals on human, organisms, and environmental health, by focussing on the interaction between disciplines and effects on the total environment. The case scenario of the wastewater discharge of Stavanger, the 4<sup>th</sup> largest city in Norway, is presented as an example of evaluation of the presence and effects of pharmaceuticals in the marine environment, combining disciplines like chemistry, biology, modelling, environmental economics and social science.

The modelling of the wastewater treatment plant (WWTP) discharge was carried out using DREAM (Dose related Risk and Effect Assessment Model). Water from the WWTP (as composite samples) of the inlet and outlet, seawater and sediment from the discharge point, as well as biota samples were collected and analysed for their chemical content in terms of pharmaceutical compounds. In parallel, laboratory exposures were carried out to better understand the potential transfer of pharmaceuticals from water and sediment to biota.

Chemical and biological analyses were conducted to quantify the risk. The collaboration with economists with expertise in environmental valuation and bioeconomic modelling helped to produce an auxiliary economic analysis relevant for stakeholders, public resource managers, and policymakers. Database of relevant environmental benefit-transfer values were compiled.

Wastewater treatment plant discharges pose a risk to the ecosystem and the organisms living in, which can become a threat to human. The One Health approach allowed a holistic vision of the environmental challenge and was accurate in identified the environmental challenge in all its aspects, which is the first important step toward effective solutions when it comes to anthropogenic impacts.

#### **7.07.T-02 A Complex Problem: Hydraulic Fracturing Wastewaters on Freshwater Organisms and Human Perspectives**

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Hydraulic fracturing is a technique used globally to recover oil and gas from impermeable sources (e.g., shale, coal beds). During this process chemical additives are injected into the ground at high pressures to “fracture” rock formations yielding oil and gas. Over time mixtures of the injected chemicals (e.g., biocides, PEG), minerals associated with the fracture (e.g., metals and salts) and hydrocarbon products (e.g., polycyclic aromatic hydrocarbons (PAHs)) will flow back to the surface as a complex solution termed flowback and produced water (FPW). Spills or intentional applications (e.g., as a dust and ice suppressant) of hydraulic FPW can be devastating to freshwater environments and communities. Overall, previous work has shown decreases in reproduction, survival, and growth of many species (e.g., *D. magna*, Rainbow trout, zebrafish) but has focused largely on survival without understanding the possible implications of climate change and how this will alter toxicity. In our study, *Daphnia* were exposed to two concentrations of FPW (0.25% and 0.75%) for 48 hours, and allowed to grow in clean media for 19 days following exposure. Growth, survival, reproduction, as well as oxygen consumption, and systems level quantitative proteomic analysis showed extensive perturbation of metabolism and protein transport in *D.magna* at exposures of 0.25% and 0.75% of whole FPW effluent. In acute rainbow trout exposures, bioaccumulation of the PAH phenanthrene was altered under different temperatures (4, 13, 17°C) and when present in combination with FPW exposure. Overall, these data will help to understand real-world exposure scenarios, and the possible biological and ecological impacts on human and ecosystem health.

#### **7.07.T-03 Effects of the Plasticizer Diethyl Hexyl Phthalate on Physical Fitness: A One Health Case Study of Contaminants at the Human-animal-ecosystem Interface**

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Environmental contamination with plastics has become a global issue impacting both high density urban and low density remote/rural environments. As a result, exposure to plastics and their component plasticizers has become a ubiquitous issue across the human-animal-ecosystem interface. These exposures can come directly from leaching of plasticizers into food and water sources, or indirectly through leaching from micro/nano plastic materials in the diet. While plasticizers have been implicated as obesogens in humans, they may also pose a threat to aquatic animals who may experience exposure through water and food sources. For example, phthalate plasticizers are suspected to influence obesity through dysregulation of the peroxisome proliferator activated receptors (PPAR), which are nuclear receptors involved in the synthesis, metabolism, and storage of lipids in the gastrointestinal system and other organ systems throughout the body. However, little research has examined whether these same pathways may be impacted in aquatic organisms and whether disrupted molecular pathways result in phenotypic responses. Recently our team has examined long term exposures to the plasticizer diethyl hexyl phthalate (DEHP), one of the most commonly found plasticizers in food and water around the world. We found that it causes distinct changes in gastrointestinal function at the molecular level, which are exacerbated by poor diet. Further, we have demonstrated for the first time that exposure to the plasticizer DEHP impacts lipid mobilization during exercise, which limits the ability to perform prolonged exercise, which can impede weight loss in humans and ecological fitness in fish. Results from these studies indicate that plasticizers have the potential to impact both humans and aquatic animals, necessitating a one health approach for dealing with this complex and global problem.

#### **7.07.T-04 Contaminated Sites and Indigenous Peoples in Canada and the USA - A One Health Investigation**

**Nil Basu and Katie Chong, McGill University, Canada**

Indigenous communities in Canada and the USA are disproportionately exposed to contaminated sites, often due to industrial activities adjacent to Indigenous lands. Contaminated sites pose unique challenges to many Indigenous peoples who consider the land as an integral part of food systems, language, culture, and spirituality. Federal management of contaminated sites near ‘fence-line’ Indigenous communities is challenged by epistemological differences, regulatory barriers, and a lack of scientific research to support community-specific exposure assessment. While pollution in general has been well-studied in the context of

Indigenous peoples, there is limited information available on the unique issue of federal contaminated sites, and a lack of coordination between industries, governments, scientists, and Indigenous communities. Most studies are focused on a local scale, which are important, but evidence synthesis is also needed to inform decision-making. Thus, we conducted a scoping review to identify and map available information on contaminated sites and Indigenous peoples in Canada and the USA, utilizing three streams of data retrieved from January-March 2022: a systematic literature search (key word groups: *Indigenous people and contaminated sites*); a grey literature search; and an analysis of federal contaminated site data (Canada's Federal Contaminated Sites Inventory (FCSI) and US EPA's Superfund Database). Our search yielded 49 peer-reviewed articles, 17 grey literatures, and 8114 federal site records (1236 Superfund, 6878 FCSI), allowing us to summarize the state-of-the-science on the exposure of 'fence-line' Indigenous communities to contaminated sites, management strategies, and the inclusion of Indigenous peoples in site processes. The results spanned many disciplines, revealing the contamination of the lands of 815 distinct Indigenous tribes and nations and the presence of 440 different contaminants or contaminant groups found at 4976 contaminated sites. By integrating three diverse data streams we discovered a disparate body of information, pointing to the need to prioritize holism, efficiency, and Indigenous leadership in site assessment, management, and research. This should include re-thinking community-specific risk assessments to better understand Indigenous conceptualizations of human and ecological health, and greater collaboration between the scientific community, Indigenous leadership, and federal governments.

**7.07.T-05 Chemical Hazard in Edible Insects – Accumulation and Elimination of Contaminants from the Substrate**  
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Considering the global food crisis, there is a need to find innovative, safe and sustainable solutions for the current food demand. At the same time, waste generation is a severe problem incompatible with the planet's carrying capacity. Insects are presented as one of the most sustainable alternative solutions as protein sources for food and feed. Due to their efficient bioconversion capacity, insects can be used as waste control, with the advantage of reusing non-valuable products and being a valuable protein source. However, while we aim for sustainable food production, safety assessments are mandatory, such as understanding how safe the use of insects is for food/feed when reared in different substrates. This study aims to go beyond the current state of the art by comprehensively evaluating how and to which extent insects uptake and eliminate contaminants over time, rather than simply measuring contaminants after exposure to a contaminated substrate. With complete two-phase bioaccumulation studies with an uptake (contaminated sediment) followed by an elimination (clean sediment) phase, data was used in toxicokinetic modelling. Identifying the key gaps in the literature, this work aims to evaluate the bioaccumulation capacity of mercury (Hg) - already reported to bioaccumulate in insects, yet without information on the elimination - and benzo[a]pyrene (B[a]P) - still not addressed as a chemical hazard in edible insects - understanding how it is uptaken and eliminated in *Tenebrio molitor* (YMW) when exposed to a maximum allowed concentration of each one of these compounds in feedstuff.

This study demonstrated that when insects are exposed to Hg and BaP at the maximum allowed levels by European Union regulation for feed, they accumulate contaminant levels that make them unsuitable for feeding other animals. However, after a depuration period, it was demonstrated that insects could reduce their contaminant to concentrations that comply with the legislation in force. In the case of Hg, 4 to 5 days should be enough for a safer consumption of these insects – a longer period is required for B[a]P. This is especially pertinent when insects are used as converters of waste, where some concerns are raised regarding all the contaminants that can be present. In those cases, this study supports the option of a depuration period in a clean substrate after exposure to the waste. This period will vary depending on the insect species and the classes of contaminants.

## **7.07.P One Health: Ecotoxicology at the Human-Animal-Ecosystem Interface**

**7.07.P-Th408 Mercury Exposure to Humans from the Consumption of Small Cetaceans in St. Vincent & the Grenadines, West Indies**

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St. Vincent & the Grenadines (SVG) has a small whaling operation, taking on average over 400 odontocetes (toothed whales and dolphins) each year for human consumption. Muscle and blubber are sold through commercial vendors, whereas liver and kidney are distributed through informal networks. Odontocetes have high tissue mercury (Hg) concentrations due to their long life spans and high trophic positions, yet little is known about Hg concentrations in Caribbean odontocetes taken for human consumption and the frequency at which they are consumed. This study 1) investigated the total Hg (THg) concentration in muscle, blubber, kidney, and liver of killer whales (*Orcinus orca*), false killer whales (*Pseudorca crassidens*), short-finned pilot whales (*Globicephala macrorhynchus*), Risso's dolphins (*Grampus griseus*), and dolphins from the *Stenella* genus taken for human consumption using a Direct Mercury Analyzer, and 2) determined the role of odontocete-based food products in the diet of the Vincentian population as a proxy for Hg exposure using interview surveys. For all species combined, mean THg concentrations (µg/g dry weight) were greatest in liver (730; n = 17), followed by kidney (274; n = 10), muscle (76.4; n = 59), and blubber (4.57; n = 36). All liver samples and most kidney, muscle, and blubber samples exceeded the FAO/WHO human consumption advisory level of 1 µg/g wet weight. Interview surveys (n = 921) determined that odontocete-based food products are consumed by 66% of respondents in SVG with meat being the most popular food product. Over a quarter (27.7%) of respondents consumed odontocetes more than once per month and 2.9% more than once per week. Based on the FAO/WHO provisional weekly tolerable weekly intake (PTWI) guidelines for methylmercury (MeHg) exposure, assuming only one of these tissues is consumed and there is no other source of MeHg exposure, blubber can be consumed the most each week (1331 g), followed by kidney (364 g), liver

(104 g), and muscle (52 g). The consumption of odontocete-based food products may present a public health risk in SVG due to the frequency at which it's eaten. It is recommended that the SVG government issue a Hg advisory regarding the consumption of odontocete-based food products and implement species-specific whaling quotas to reduce the likelihood of the most Hg contaminated species (killer whale and short-finned pilot whale) being consumed.

#### **7.07.P-Th409 Implications of Climate Change for Dietary Contaminant Exposure in Alaskan Subsistence Communities**

**Kristin Nielsen**, *University of Texas at Austin*

Remote coastal communities in high latitude regions are especially vulnerable to adverse impacts of climate change on community health and resilience. This is particularly evident in Alaskan subsistence communities, where changes in the timing, abundance, availability, and quality of subsistence resources are contributing to a range of concerning public health inequities and cultural erosion. Rural Alaskans have some of the highest fish consumption rates in North America; however, subsistence consumption of fish has declined by 31% since the mid-1980's. Community surveys indicate that these trends are driven by a few primary factors - notably, climate change and pollution, which are inextricably linked by chemicals with global biogeochemical cycles that are tightly coupled to climate (including some contaminants). Changes in various climate-driven factors (e.g., melting permafrost, diminishing sea ice, glacial retreat) may necessitate changes to traditional food storage and preservation methods and meaningfully alter the composition of subsistence diets via several direct and indirect mechanisms, both of which have implications for dietary contaminant exposures. This presentation will discuss climate-driven factors that may contribute to food safety and security issues in rural and Alaska Native subsistence communities, as well as the relative risks to public health that are attributed to increasingly westernized diets in high-latitude subsistence communities in Alaska.

#### **7.07.P-Th411 Microplastics in a Local Population of Dolphins (*Tursiops truncatus*): A One Health Approach to Coastal Monitoring**

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Within the tidal creeks of Charleston, South Carolina (USA) resides a population of bottlenose dolphins (*Tursiops truncatus*) that represent sentinel species for monitoring the health of coastal ecosystems. Microplastics (particles less than 5mm) are anthropogenic contaminants of emerging concern that are ubiquitous in the environment and have been documented within marine biota, including in piscivorous predators such as dolphins. However, no long-term studies have assessed microplastics in a local population of dolphins. This research aims to quantify and identify microplastic particles in the gastrointestinal tracts of bottlenose dolphins stranded around the Charleston estuary dating back to 2016. We examined dolphin stomach and intestinal contents for microplastics by rinsing contents into stainless-steel sieves to retain particles  $\geq 125\mu\text{m}$ , digesting biological remains with a 10% KOH solution, and using Raman spectroscopy to identify polymer types. Preliminary results from seven stranded dolphins indicate high levels of contamination, ranging from 76-2249 total microplastics per animal ( $1175 \pm 288$ , mean  $\pm$  SE). The most common plastic polymer identified was polypropylene (35%), which is commonly used in fishing gear. This ongoing research will continue to assess archived dolphin samples and also investigate levels of microplastics in local fish to elucidate the potential for trophic transfer of these contaminants into the diets of mammals, including humans. This study can help inform coastal managers by taking a One Health approach to assess the health of our coastal ecosystem, animals, and also humans.

#### **7.07.P-Th412 A One Health Solution to Control Residual Malaria Transmission: The Not-So-Hidden Added Value of Ecotoxicology and Environmental Chemistry**

**Andre Patrick Heinrich<sup>1</sup>**, *Hermann Sié Pooda<sup>2</sup>, Christophe Roberge<sup>3</sup>, Rolf-Alexander During<sup>4</sup> and Karine Mouline<sup>5</sup>*, (1)Institute of Soil Science and Soil Conservation, Justus Liebig University Giessen, Germany, (2)Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES), Burkina Faso, (3)MedinCell, France, (4)Justus Liebig University Giessen, Germany, (5)Institut de Recherche pour le Développement (IRD), France

Sixty years ago, Rachel Carson's *Silent Spring* shaped environmentalism and exposed hidden ecological costs of the first modern insecticide DDT which revolutionized malaria control. Despite major environmental and health risks, DDT is still used as last resort to fight malaria vectors (mosquitoes). The burden of the disease plateaued in recent years, and it became clear that human health is closely linked to animal and environmental health, ultimately resulting in the holistic strategy called One Health.

In the combat against malaria, the success of control tools like insecticide-treated nets and indoor residual spraying is often undermined by new behaviour of mosquitoes to escape exposure. Zoophagy is one of them, with mosquitoes being diverted by control tools to alternative blood sources than humans, which are mostly domesticated animals. In Burkina Faso, the ANIVERMATE project investigates treating cattle with ivermectin as a new vector control tool. The idea: *Anopheles* mosquitoes receive ivermectin via blood meal and die. However, since excreted veterinary drugs can enter the environment and harm ecosystems, ecological consequences of residues are considered upfront. Combining human, animal and environmental health creates a true One Health approach. Showcasing the expertise and added value that ecotoxicology and environmental chemistry bring to the table of human health concepts was the main motivation of this One Health project.

Our 3-arms design study used local cattle and *An. coluzzii* mosquitoes from Burkina Faso. We compared a classical, injectable ivermectin veterinary formulation (IVOMEC-D®) and a new long-acting depot formulation designed to provide vector control for at least six months. First, we characterized the pharmacokinetics in cattle plasma and dung. Efficacy was evaluated relatively to ivermectin plasma concentration by following mosquito mortality after direct skin feeding assays on treated cattle hosts at different delays after application. Modelling approaches were used to determine efficacy in the field setting.

To assess environmental risks, we monitored ivermectin dissipation in dung over a 90-day period. Sorption studies complemented this environmental fate review. The predicted environmental concentrations and behavior were related to published ecotoxicological observations on dung fauna. Kinetics in dung and plasma and the preparatory environmental risk assessment are presented together with proposed risk mitigation measures.

#### **7.07.P-Th413 Distinctive Gastric Structural Responses of Wistar Rats Exposed to Environmental Concentrations of Tributyltin (TBT)**

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For decades, the application of antifouling (AF) such as tributyltin (TBT) has been used not only for its antifouling feature but also in other industrial processes. In consequence, tons of this compound are still available in aquatic environments. As water treatment systems are still very deficient in eliminating persistent and highly toxic compounds, their presence in nature affects not only organisms in general, but also human health. Therefore, this study aimed to evaluate the histopathological effects of the gastric structures of Wistar rats exposed to environmental concentrations of TBT. For that purpose, 36 male Wistar rats were used. The experiment was carried out in 60 days (photoperiod of 12HL/12HD, 23°C±1°C), the rats were divided into 3 experimental groups with 4 animals per group: group (CG), the control group, by gavaging only with corn oil; group 1 (G1), gavaged with 20ng.g<sup>-1</sup> of TBT diluted in corn oil; and group 2 (G2), gavaged with 600ng.g<sup>-1</sup> of TBT diluted in corn oil. Then, the organisms were euthanized by the decapitation. The stomach of each group was fixed in formalin solution 10%, then the materials were proceeded under histological routine. Cuts of 5µm were stained with Hematoxylin-Eosin; Picrosirius Red; Masson Trichrome; Periodic Acid Schiff and Alcian Blue pH 1.0. The histological morphometric parameters and analyzed by Tukey's One-Way ANOVA. The G1 presented the stomach with several structural and composition damages. The nonglandular stomach presented thick layer of keratin. The hyperkeratosis can be related to direct contact with TBT, leading to inflammation, ulcer and cancer. The mucus secretion was poorly seen, resulting in malformation of bolus. Muscular layer atrophy was severe in this treatment. In the G2, the nonglandular stomach had hyperkeratosis as seen in G1. The glandular stomach mucosa was poorly in mucus secretion. In all strata, there were hemorrhagic centers infiltrated lymphocytes mainly in the submucosa, indicating inflammatory process. In the long-term, the gastric function can be damaged and disabled. We conclude that the ingestion of TBT even at environmental concentration has significative changes in the gastric morphophysiological composition. The TBT can affect mainly the aquatic organisms, due to accumulating and affecting the trophic chain, so using a rat as a human parallel organism, we can measure the morphophysiological damages of environmental levels of TBT in our food and water.

#### **7.07.P-Th414 Can I Eat This Fish? - Dietary Recommendations to Reduce the Mercury Risk Exposure in Portugal**

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Fish consumption is undoubtedly an important component of the human diet, with recognized nutritional value. Unfortunately, fish consumption is also a major pathway of human exposure to mercury (Hg).

In recognition of the risks associated with exposure to Hg through diet, the Joint Expert Committee on Food Additives (JECFA) have determined a maximum limit to Hg intake called provisional tolerable weekly intake (PTWI) of 1.3 µg kg body weight (bw)<sup>-1</sup> week<sup>-1</sup>. Furthermore, JECFA also established the maximum permitted Hg level of 0.5 µg g<sup>-1</sup> (ww) in fish muscle and 1 µg g<sup>-1</sup> (ww) in the muscle of some predatory fish. Despite that, JECFA concluded that determining reference levels for Hg in fish may not be the best way to reduce Hg exposure in the general population. On the contrary, it recommends advising risk groups on the risk and benefits of fish consumption in order to effectively reduce the number of individuals with higher exposures than PTWI. Fish consumption in Portugal increased 9% from 2015 to 2018, reaching 60.9 kg in 2018 (36.6 kg higher than EU-28 average). Portugal is thus one of the countries with the highest fish consumption per capita in the EU and the world. Interestingly, other countries with lower fish consumption per capita (e.g. USA, Canada, Ireland, Australia and New Zealand) have already established specific guidelines considering the risk-benefit of fish consumption, whereas, in Portugal, such guidelines are lacking. Despite some scientific publications focusing on Hg exposure through fish seafood consumption, the results of these studies have not yet been applied in the development of specific recommendations. A knowledge transfer between the academia and the society would empower the population with tools to a responsible selection of the fish species and amount of fish to eat considering the risk-benefit of fish consumption.

So, in order to bridge this gap, CAN I EAT THIS FISH? project aims to: 1) provide the largest and more complete assessment of Hg levels in fish in Portugal considering different ecological and geographical aspects (with a special focus on the Estarreja Region, due to the scenario of historical contamination by Hg). 2) evaluate the exposure of the Portuguese population to the Hg through diet. 3) develop tools with simple and precise language that allow mitigating exposure to Hg. An overview of the project's main aims and tools to achieve the proposed objectives will be discussed in detail.

#### **7.08 Precision Application – A Way to Reduce Environmental Risk?**

##### **7.08.T-01 Digitalization - Chances and Risks of New Technologies in Plant Protection for the Environment and Sustainability**

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The aim of the presented study was to describe the landscape of developments in new plant protection technologies and strategies, and identify their potential for reducing the environmental impact of plant protection in practice. Not only the opportunities and risks of new technologies need to be evaluated, also their maturity for implementation into agricultural practice must be estimated.

Current developments were identified by means of literature research and qualitative interviews and selected for their potential impact. A stakeholder workshop was organized to present the outcomes of the survey and discuss the results.

The highest level of maturity of some spray application systems was confirmed during the stakeholder workshop. Most participants voted for spot spraying, followed by band spraying, weeding robots and decision-making support. A high potential to reduce the impact of plant protection on the environment was seen for robot technologies.

The results indicate, that particularly techniques related to patch treatments show a high potential to decrease the impact on the environment, especially when connected to an alternative to pesticide application. The stakeholder workshop made clear, that further development is needed for sensor-based strategies respecting in-field biodiversity.

Cited obstacles for the implementation of the technologies were unknown rentability, deficits in infrastructure and complexity of data exchange interfaces. The demand for data to measure the progress in sustainability confronts with the fear of control and sanctioning. As stated in the workshop, it is very much the personal motivation of the farmer that decides on the implementation into practice and the potential impact on the environment.

For the implementation of any development into practice the stakeholder workshop recommended comprehensive and independent advice and effective financial support at the farm level, financing the application and achievement of environmental goals, not just the financing of a new device.

As the digital transformation concerns all crop management areas, it is reasonable to regard risks and opportunities on the farm level and look out for synergistic effects, especially for the investment and environment. Since the farmer plays a key role in the adaptation of the new technologies, measurement and evaluation of the environmental effects should be possible on the farm level as feedback and incentive, not only in research and regulation.

#### **7.08.T-02 Exploring Groundwater Leaching Concentrations From Partial-Area Applications: A Two-Dimensional Modelling Exercise**

*Francis P. Donaldson and Bernhard Jene, BASF SE, Germany*

Agrochemical leaching in the EU is assessed for regulatory purposes with one-dimensional transport models which exclusively consider vertical transport to underlying groundwater. Such an approach is reasonable for homogenous broadcast field applications, as lateral dispersion and transport are mostly only minor effects as they are only relevant at the boundaries of the sprayed area (*i.e.*, the field boundaries). However, such transport processes may become more important in the case of targeted and precision partial area application techniques (such as banded and spot application) as most of the applied area would become influenced by a boundary between sprayed and unsprayed areas.

Two-dimensional modelling work using HYDRUS-2D has been previously presented which demonstrated the concentration reduction in groundwater when considering a banded application. The present work further expands on this effort, comparing one-dimensional (vertical transport) modelling with more realistic two-dimensional modelling approaches. The impact of input parameters (including nonlinear sorption) is considered, and comparisons across all FOCUS groundwater scenarios are made to demonstrate that the groundwater concentrations will be affected (and scale) with the fraction of the treated area.

#### **7.08.T-03 Precision Application and Soil Organisms – Matter of Scatter or Just Chatter?**

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Technological innovations such as precision spraying makes it possible to treat agricultural fields partially or individual plants only. This leads to heterogeneous pesticide spraying patterns compared to conventional full-area application. In turn, soil organisms are exposed to a correspondingly heterogeneous load regarding the field perspective. However, how such 'precise application' of pesticides affects soil organisms on the field level is very limited. In the context of precision application pattern, it is not the absolute amount of agent applied per field what is considered crucial, but the actual spatial distribution of compound and soil organisms across an agricultural field. It is assumed, that the pattern itself has a strong influence on how individual species, functional groups and the community are affected. For this reason, a study was designed to investigate the effects of a precise spray application on the earthworm community within a field scale. Carbendazim was applied at an application rate of 8 kg a.s./ha on 50% of the treatment area in 3 different checkerboard application patterns with different scaling. In addition, a fully applied positive and a negative control were carried out. The earthworm community was observed over a period of one year. In addition to total earthworm abundance, different ecological groups were analyzed separately. Our results show that primarily the scale of the application pattern has a clear influence on total earthworm abundance with different ecological groups showing different responses. The results of the study contribute to a better understanding of the possible effects of precision application on different ecological groups of soil organisms and will be presented and discussed.

#### **7.08.T-04 Modelling Spray Drift in Precision Applications**

*Henk Jan Holterman, Koen Van Boheemen and Jan Huijsmans, Agrosystems Research, Wageningen University & Research, Netherlands*

Precision application of plant protection products (PPPs) results in a reduction in the amount applied and could also result in a lower exposure of different off-field areas and non-target organisms to PPPs. Precision applications for crop protection could play

a role in the exposure assessment of PPPs in the authorisation process. Typically, variable-rate and spot-spraying applications can be distinguished. In a variable-rate application the whole field is treated while the dose is varied to meet the conditions in each location. In a spot-spraying application for each location the decision is made whether or not a (full dose) spray application is required. Finally, a hybrid form exists where variable-rate and spot-spraying are combined. This study focusses on the exposure reducing effects of variable-rate and spot-spraying applications in arable crops, particularly regarding the downwind off-field deposits due to spray drift. For this purpose a new spray drift model was developed which takes into account the non-uniform dose rates at the crop field. In the model, field sections receiving a certain PPP dose rate are identified as polygons. This approach is similar for both variable-rate and spot-spraying applications. Implicitly, each polygon is treated as a (very) small crop field. As such, each polygon contributes to downwind spray drift, depending on its area and on the applied dose rate. The sum of all these contributions is the total spray drift that occurs. In the current set-up, adjacent watercourses are the non-target areas to protect. Watercourses are imaginarily divided into segments of about 10 m length. Spray drift deposits are computed on each watercourse segment. Spray drift deposits on a watercourse segment depend on its location with respect to the treated field sections and on wind direction. Only downwind segments can receive spray drift deposits. Variable-rate and spot-spraying applications lead to drift reduction, partly due to the reduced amount of PPP used. Example computations indicate that drift reductions can be higher or lower than the reduction from reduced amount of PPP. This depends on the distribution and location of field sections with reduced dose rates. In this study, a few test cases are investigated for different wind directions. The results indicate that drift reductions seem to be rather insensitive to wind direction. Further research is needed to verify this remarkable finding.

#### **7.08.T-05 DriftRadar by Bayer: Integrated Drift Management During Spray Operation to Prevent Drift**

**Andrew C. Chapple<sup>1</sup>, Josy Bravidor<sup>2</sup>, Frank Dietz<sup>3</sup>, Yves Jaschinski<sup>3</sup>, Susanne Lauck-Birkel<sup>3</sup>, Catherine Legue<sup>4</sup>, Walter Mayer<sup>3</sup>, Daniela Portz<sup>3</sup>, Robert Puhl<sup>3</sup>, Katarzyna Maria Ubyasz<sup>5</sup> and Anna Zdunek<sup>5</sup>,** (1)Environmental Safety, Bayer AG - Crop Science Division, Germany, (2)Bayer AG - Crop Science Division, Eritrea, (3)Bayer AG - Crop Science Division, Germany, (4)Bayer S.A.S., France, (5)Bayer Sp.z.o.o., Poland

DriftRadar, an ongoing project at Bayer AG, is a stepping-stone towards a vision of a fully integrated digital label with digitalized, *in situ* or field-localized risk assessment and customized risk mitigation in real-time. It demonstrates a solution to increasing label comprehension (*c.f.*, French national, department, and local drift requirements), sustainability, and regulatory compliance by implementing the right label recommendations for selected products applied to a crop using automated spray application. DriftRadar helps to manage the complexity of dealing with multiple risk assessment compartments for vulnerable off-target areas (*e.g.*, a residential area downwind or a stream next to a cropped field) and can consider the conditions in the field – the wind direction. Label requirements are loaded from a regulatory compliant database (the example used here is Bayer's LabelHub) and automatically applied downwind to the adjacent areas in a customized way during spray operation. Off-crop (NTA/NTTP, water, residential areas and adjacent fields) are mapped into the software. A GPS sensor captures the exact position of the sprayer in the field and a wind sensor measures wind direction every 10<sup>th</sup> of a second. These parameters are used to close nozzles or change to drift-reducing nozzles while spraying and the required no-spray buffer-zones (*e.g.*, towards residential areas) are automatically applied during operation to prevent drift (*e.g.*, to bystanders). Software records all activities including the *in situ* wind direction at the scale of the decision making and finally stores this data as part of the normal record-keeping activity on the farm in compliance with regulations. Two proofs of concept for this approach have been successfully completed at the ARVALIS research station at Boigneville, France and at Inhort, Skierniewice, Poland, and have been shown to the French and Polish regulatory authorities. Improvements to the system as suggested by the research institutes are being implemented. Through the work described, we have demonstrated the possibility that, eventually, all aspects of the regulatory oversight of pesticide spray application can follow a digitalized, *in situ* or field-localized risk assessment. It also opens up the possibilities of integrating customized risk mitigation in real-time for local field conditions. [A 360° preview of the system can be seen at <https://go.bayer.com/driftadar>.]

#### **7.08.P Precision Application – A Way to Reduce Environmental Risk?**

##### **7.08.P-We438 How Do We Develop Environmental Risk Assessments for the Precision Application of Herbicides ?**

**Joanna Davies, Syngenta, United Kingdom**

Regulatory guidance updates continue to restrict the use of herbicides in the EU while the EU Green Deal has set the target of reducing pesticide volume and risk by 50% by 2030. Specifically, proposed changes to risk assessment scheme for non-target terrestrial plants may prohibit the broadcast use of some products completely. Work conducted on behalf of CropLife Europe has demonstrated that in the absence of herbicides, growers may adopt alternative weed control practices, notably mechanical weed control. Mechanical weed control has unintended adverse consequences on several economic and environmental factors, including food production costs, machinery and fuel usage, soil health, surface water quality (via increased nutrient run-off) and, ultimately, climate change. These adverse effects run contrary to the principles of regenerative agriculture, which promotes retention of carbon within soil and aims to limit the contribution of agriculture to climate change.

Precision application (PA) technologies for herbicides provide a means of reducing total volumes applied per unit area, whilst avoiding the need for mechanical weed control. However, current EU risk assessment approaches assume application at a maximum broadcast application rate to 100% of field area. In cases where treatment of entire fields is not necessary for weed control, new risk assessment approaches are needed to take account of the reduction in treated area. The consequences of these reductions for risk assessment will vary for each environmental compartment, (*i.e.* soil, ground water and surface water) and non-target organism (NTO) class (*i.e.* birds, mammals, aquatic organism, bees, non-target arthropods, soil organisms and non-target plants). Faupel et al suggest that some aspects of risk assessment, notably groundwater, drainage-driven exposure and mobile

NTOs, can be adapted by assuming that a reduction in treated area is equivalent to a corresponding reduction in broadcast rate. However, for risk assessments that are driven by drift estimates or less mobile NTOs, new approaches are needed that take account of the spatial and temporal distribution of the application across a field.

To inform the development of environmental risk assessments for the PA of herbicides, the following factors are considered in this presentation: (1) PA systems and capabilities; (2) spatial and temporal distribution of weeds (3) relevance of PA system and weed distribution to environmental assessment.

#### **7.08.P-We439 How to Consider the Benefits of Precision Application in Regulatory Risk Assessment**

**Rena Jutta Irene Isemer<sup>1</sup>, Joachim Dressel<sup>2</sup>, Francis P. Donaldson<sup>3</sup>, Thomas-Georg Hofmann<sup>3</sup>, Anne Alix<sup>4</sup>, Laurent Oger<sup>5</sup>, Sophie Hane-Weijman<sup>6</sup>, Martin Herchenbach<sup>7</sup>, Neil Morgan<sup>8</sup> and Bernhard Gottesbueren<sup>3</sup>,** (1)Crop Science, Bayer AG - Crop Science Division, Germany, (2)Syngent Crop Protection AG, Switzerland, (3)BASF SE, Germany, (4)Corteva Agriscience, United Kingdom, (5)CropLife Europe, Belgium, (6)FMC Corporation, Switzerland, (7)Digitalisation, Industrieverband Agrar e.V., Germany, (8)Syngenta, United Kingdom

In the European Union, Plant Protection Products (PPP) are subject to thorough risk assessments prior to registration. These risk assessments require that exposure estimates reflect “worst-case” scenarios built on conservative assumptions in order to cover all conditions of cultivation for a particular crop across Europe. Consequently, the risk assessment almost never represents the circumstances under which crop protection products are actually applied. The development of digital and precision tools as well as targeted application equipment, enabling that the most appropriate use rate is applied only where needed, is underway. These will become the way to use products in the next decade, as per the Green Deal objectives. Combining existing spatial information with the additional data generated by these innovative technologies, site-and-time specific risk assessments will become possible. It therefore becomes necessary to reconcile risk assessments with the application practices in use in the field.

Regulators at national and EU level as well as industry experts are working to address how digital and precision application (PA) can be taken into account in risk assessments. In order to facilitate and enable the activities necessary to integrate the required changes into the regulatory risk assessment, CropLife Europe formed the Digital Ag in Regulatory (DARE) team in 2021. A roadmap has been developed, demonstrating how the EU approval procedure for PPPs should be adapted over time: 1) Adaptation of existing assessments for PA uses with new data and concepts, as well as with risk mitigation measures reflecting capabilities of PA tools. 2) Introduction of new risk assessments for PA uses into guidance documents. 3) Development of the regulatory paradigm towards execution of risk assessments and risk mitigation for PA uses based on actual field data at the time and location of application, depending on data availability and capabilities of the technology, supported by electronic documentation.

The team has initiated a mapping of relevant activities across EU institutions, Member States, and industry associations, which will be presented. A proposal will be made on how precision application could be included in the current regulatory framework for specific areas. Working objectives for the scientific/regulatory community to foster the inclusion of precision applications and its many benefits in the risk assessment will be proposed.

#### **7.08.P-We440 Spotted: Heterogenetic Pesticide Exposure and its Effect on Springtails**

**Melanie Hagen-Kissling<sup>1</sup>, Elias de Bree<sup>2</sup>, Rik Delhem<sup>2</sup>, Livia De Felici<sup>2</sup>, Micha d'Oliveira<sup>2</sup>, Julia Friman<sup>2</sup>, André Grove<sup>2</sup>, Silvio Knaebe<sup>3</sup>, Koen Verhoogt<sup>2</sup> and Bogdan Dehelean<sup>2</sup>,** (1)Eurofins MITOX B.V., Netherlands, (2)Eurofins-MITOX B.V., Netherlands, (3)Eurofins Ecotox, Germany

Precision farming, i.e., applying pesticides precisely to field areas of pest infestation only, is a promising method to decrease the negative effect of agricultural production on the environment, without decreasing crop quality at the same time. So far, it is not known what the effect of such heterogenic pesticide exposure on the dynamics of soil arthropods, especially those with small home ranges would be. Here, we focus on springtails (Collembola), which are among the most abundant groups of soil decomposers, inhabiting various organic substrates. Furthermore, they are a food source for a wide range of specialist and generalist predators, making them a key group for the functioning of ecological networks.

Here we ask: How does a patchy application of insecticides translate into effects on springtail populations and communities? Is the time until recovery after a pesticide application in springtails correlated/depending to/on (1) direct overspray, (2) the proportion of treated surroundings, (3) the distance to treated surroundings?

To answer these questions, we conducted a field experiment to assess the effects of patchy pesticide spraying on springtail populations and communities. The study was performed in a winter wheat field, using a checkerboard design. This design consisted of control plots and treatment plots sprayed with an increasing proportion of pesticide (20%-100%). Springtails were monitored using suction sampling technique. Data analysis was performed both at the level of the arthropod community and at the level of the individual populations.

The knowledge about the effect of patchily sprayed fields on the dynamics of springtail populations is crucial to compare effects of patchy versus continuous application of a pesticide on springtails. Furthermore, the gained information could be used to populate spatially explicit population models, which can help to explore effects of heterogenic pesticide exposure on arthropod populations on larger spatial scales.

#### **7.08.P-We441 Precision Agriculture – Implications for a Bird and Mammal Risk Assessment Under Regulation 1107/2009**

**Joerg Hahne<sup>1</sup>, Markus Ebeling<sup>1</sup>, Manousos Foudoulakis<sup>2</sup>, Steven Kragten<sup>3</sup>, Alex Blakey<sup>4</sup>, Joachim Nopper<sup>5</sup>, Thomas Bean<sup>6</sup>, Dennis Sprenger<sup>7</sup>, Sonja Haaf<sup>8</sup> and Alex Mauss<sup>1</sup>,** (1)Environmental Safety, Bayer AG - Crop Science Division, Germany, (2)Corteva Agriscience, Greece, (3)Syngenta Agro, Germany, (4)Environmental Safety, Syngenta, United Kingdom, (5)BASF SE, Germany, (6)FMC Corporation, (7)Corteva Agriscience, Germany, (8)Environmental Safety, ADAMA Deutschland, Germany

Since the adoption of the current Sustainable Use Directive (SUD) in 2009, technological advancement led to a significant

number of new digital and precision agricultural solutions, including precision application of pesticides. Using plant protection products to a level of agronomic need is in line with the objectives of the 'European Green Deal' to reduce pesticide use by 50% by 2030. Although the existing regulatory framework already provides a high level of protection against possible detrimental effects on the environment, the use of precision agriculture can further reduce exposure and consequently risk. Precision agriculture is defined as "doing exactly what a plant or animal needs within the limits of time and space and according to economic and social boundaries with respect for environmental aspects". Practical examples indicate that, e.g., variable applications can currently reduce the applied plant protection product by between ~10 and ~40%. To reflect precision agriculture also in the regulatory decision making on plant protection products, an understanding of which practices qualify as precision agriculture and lead to reduced environmental exposure is needed. Guidance is currently lacking on how to account for these practices in the risk assessment for birds and mammals, where precision agriculture techniques might help reduce exposure, particularly for long-term risk assessments.

We will highlight four different precision application techniques for pesticides (precision drilling, shielded band spray, drip irrigation and spot application) that can reduce field exposure to birds and mammals. We identify for each of these techniques open points in the current bird and mammal risk assessment scheme and propose possible solutions on how these could be addressed. To develop specific bird and mammal risk assessment schemes for precision application and to discuss how to address open questions a dedicated workshop involving relevant stakeholders might be needed.

#### **7.08.P-We442 Spatially Explicit Population Models Support Impact Assessment of Precision Application – A Common Vole Example**

**Oliver Jakoby**, Torben Wittwer, Felix von Blanckenhagen, Tina Grimm, Lara Ibrahim, Martin Vallon and Michael Faupel, RIFCON, Germany

Precision farming has the potential to reduce the impact of pesticides on the environment. Particularly, a more precise use of pesticides can result not only in a reduced pesticide load, but also in a spatially heterogeneous application pattern on the field scale. Accordingly, the effect of the pesticide on populations of non-target species might change. Spatially explicit effect modelling is a valuable tool for environmental risk assessments (ERA) of pesticides, which can also be applied when considering precision applications. Models allow a practical and cost-effective investigation of a multitude of application and exposure patterns leading to the understanding of their effects on non-target in-field species populations, the quantification of uncertainties and accordingly, they have the potential to optimize precision application patterns even further.

RIFCON's individual-based and spatially explicit population model *eVole* is frequently used in ERA for small mammals to understand the risks of pesticide applications on a field to landscape scale. In this hypothetical case study, we use *eVole* to investigate the impact of spatially heterogeneous pesticide applications in agricultural fields on common vole (*Microtus arvalis*) populations. Specifically, different schematic pesticide application patterns, including band applications as well as spot and patch treatments, are tested and compared to a standard full field application of the pesticide.

Differences in the magnitude and duration of potential effects on vole populations are quantified and the impact of differently fragmented precision applications are discussed. In addition, the proportion of food taken from treated habitat per individual are extracted and the differences in the distribution over the whole population are examined.

Our case study demonstrates the potential of spatially explicit effect models in investigating, understanding and optimising precision applications. The results provide cost-effective information that can improve the overall understanding of the underlying mechanisms to support risk assessment and management, as well as complement field study approaches.

#### **7.08.P-We443 Modelling the Reduction Effect of Spot Applications on Pesticide Runoff Losses from Fields with a PRZM-VFSMOD Coupling**

**Stefan Reichenberger**<sup>1</sup>, Robin Sur<sup>2</sup>, Klaus Hammel<sup>2</sup>, Jorge Olivares-Rivas<sup>3</sup> and Rafael Muñoz-Carpena<sup>4</sup>, (1)knoell France SAS, France, (2)Bayer AG - Crop Science Division, Germany, (3)knoell Germany, Germany, (4)University of Florida

Increasingly, crop protection products are not applied homogeneously over the whole field, but rather as so-called spot application to selected field areas. For treated spots not located directly at the downslope edge of the field it may happen that a part of the pesticide lost with surface runoff from the treated spots may be removed from the surface runoff via infiltration or sedimentation before reaching the field outlet. The objective of this study was to test the hypothesis that the untreated area between the field edge and the treated spots can reduce the loss of pesticides via surface runoff and erosion from the target area to receiving surface water bodies.

A large number of simulations with a coupled modelling system (PRZM, VFSMOD and a TOXSWA metamodel) were performed in order to quantify the effect of untreated field areas on the retention of pesticide-laden water and sediment as a function of the distance between treated spots and field edges. The spot application was modelled using a geometrically simplified approach (dimension reduction), that is, aggregating the treated spots to a band with a defined width and distance from the downslope field edge. The upslope untreated area and the treated band were simulated with PRZM (FOCUS R1 scenario for winter cereals; spring application of 8 different dummy compounds with different sorption and degradation properties). The surface runoff and eroded sediment generated by the upslope part of the field were transformed into a runoff hydrograph as input for VFSMOD, which was used to model the downslope part of the field. The parameterization of VFSMOD was adapted to reflect an annual arable crop as opposed to a permanently grassed buffer strip. For each event in the simulation period, Predicted Environmental Concentrations in surface water (PEC<sub>sw</sub>) were calculated with a TOXSWA metamodel. A large number of different widths and positions on the slope of the treated band were tested and the pesticide losses and PEC<sub>sw</sub> evaluated.

Since VFSSMOD models only sediment deposition, but not erosion, the downslope part of the field is treated as a sink area for eroded sediment, while in reality it could also be a source area. Consequently, the range of applicability of this assumption (no erosion from the downslope part) was also investigated.

#### **7.08.P-We444 Digital Label Compliance as an Enabler for Bringing Realism to Risk Assessments: A CropLife Europe Initiative**

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Plant Protection Products (PPPs) in agriculture are regulated through EC Regulation No. 1107/2009. The evaluation process imposes preliminary risk assessments and the precise setting of directions of use for the active substance at the EU level. This is followed by the PPP authorisation at the member state level with implementing risk mitigation measures before placing them on the market (EC, 2009). The risk assessments rely on hundreds of guideline-compliant studies and are performed to reflect worst-case use and exposure scenarios (EC, 2013a and 2013b). Label instructions are established based on the outcome of these worst-case scenarios used for the risk assessments. Therefore, they cannot reflect the high potential of adaptive application scenarios depending on the conditions under which products are used in the field. The diversity European farmers face, agro-environmental conditions and pest pressures faced by European farmers are also not reflected. Furthermore, modern technologies and stewardship measures are rarely considered in risk assessments even though they are essential to meet increasingly stringent compliance requirements and represent a central piece of developing a more sustainable food production model as outlined by the EC's Communication on the EU Green Deal and specifically in the F2F and the Biodiversity strategies which aim to reflect consumer, policymaker and societal expectations.

This presentation will introduce the Digital Label Compliance (DLC) project being developed by CropLife Europe, working with various stakeholders. The DLC aims to increase label comprehension and compliance via digital labels offering easy access and readability of the typically highly complex label directions, and so contribute to reducing risk during all relevant operations, including handling and application of Plant Protection Products by growers. The digital label not only enables the future automated application of Plant Protection Products according to the registered directions for use, including all precautions but also allows consideration of the geolocation and actual conditions of a grower's field. This, combined with the subsequent recording of spray operations provides a robust basis for the collection of data reflecting real conditions of use, which can feed into realistic field-specific risk assessment scenarios.

#### **7.08.P-We445 Digital Labels Enabling Field Specific Risk Assessment and Tailored Risk Mitigation**

*Karandeep Singh<sup>1</sup>, Susanne Lauck-Birkel<sup>1</sup>, Andrew C. Chapple<sup>2</sup>, Katja Timm<sup>1</sup> and Sarah Lewandowski<sup>3</sup>, (1)Bayer AG - Crop Science Division, Germany, (2)Environmental Safety, Bayer AG - Crop Science Division, Germany, (3)Bayer AG - Digital Transformation and IT Crop Science*

A typical agricultural product comes with a physical label providing important information about the safety, quality and specific information identifying the product in the container. However, there is limited space to share enriched, detailed, customized information for a given product that is useful for farmers to make data-driven decisions that support precision and enhanced sustainability. For our Plant Protection Products (PPPs), directions for use and all precautions to be taken at all stages must be indicated on the label, thus making labels increasingly more complex to read. Label instructions are established based on the outcome of the worst-case scenarios considered for the risk assessments and thus rarely reflect the actual conditions under which products are used in the field. This could include a diversity of application machinery, agro-environmental conditions, and pest/weed/fungal pressures faced by the farmers. The rapidly changing needs of farming towards digitalization and integration of all inputs and outputs into planning software to drive decisions and document them require a smooth and efficient label data transfer. Access to the relevant label data is the backbone of any such solution. We show how two ongoing initiatives at Bayer AG, "DriftRadar" enabled by the "Digital Label"- demonstrate a complete end-to-end solution to increasing label comprehension and regulatory compliance by implementing the right label recommendations on e.g., buffer zones for selected products applied to a crop using automated spray application. In order to achieve an efficient front-end application like DriftRadar, we require high quality and up-to-date relevant label data in a structured machine-readable and -actionable format. We have developed LabelHub, a new central data platform, and a central access point for a fast and flexible data provision. It collects, harmonizes and transforms data from Bayer internal and external sources. It opens up the opportunity to include additional data like regulatory endpoints required for *in situ* risk assessments and tailored risk mitigation specific to the actual field, will allow to adapt to changing risk assessment needs with new data and concepts, and will allow to execute- these assessments and risk mitigation in future based on actual field data at time and location of application, depending on data availability and capabilities of the technology.

#### **7.08.P-We446 Targeted Spray Application in Onions Using a Crop-Adapted Pulse Width Modulation Sprayer**

*Gerrit Van Steenberg and Henk Jan Holterman, Agrosystems Research, Wageningen University & Research, Netherlands*

Onions are a difficult to spray crop since its canopy is open and the leaves are pointing upwards. The major part of the sprayed liquid is sprayed onto the ground between the plants on the onion bed and on the path between the beds. A field experiment was performed to measure spray deposition on the onion leaves and on the ground of a reference sprayer, a crop-adapted pulse width modulation (PWM) sprayer and a crop-adapted PWM sprayer with a lowered sprayer boom in combination with nozzles above the paths directed towards the beds. The goal was to measure if the crop-adapted PWM sprayers are able to retain spray deposition on the crops while lowering the total sprayed volume on the field. Lowering the total sprayed volume on the field will decrease the emission of plant protection products to air, ground and surface water. Spray deposition was measured in three crop

stages when the plants had a height of around 15 cm (BBCH 13/14), 35 cm (BBCH 42) and 55 cm (BBCH 44). Before spraying, the Normalized Difference Vegetation Index (NDVI) was measured using a HandySpec handheld crop sensor. The dose rate was adjusted based on the NDVI from 0.2 to 0.7 to a rate of 90 to 300 l/ha, respectively, by changing the PWM duty cycle. Spray deposition on the leaves and on ground collectors was analysed using a spectrophotometer. The relative deposition on the onion leaves using the crop-adapted PWM sprayer was equal or significantly lower than with the reference sprayer in all crop stages. The relative deposition on the onion leaves using the PWM sprayer with lowered sprayer boom and nozzles directed towards the crop was equal in crop stage 1 and significantly higher in crop stage 2 and 3 compared to the reference sprayer. Spray deposition on the paths between the onion beds for this sprayer was 30% lower compared to the reference. A low sprayer boom and directing the nozzle above the path towards the onion beds increases relative spray deposition on the leaves in later growth stages. No increase was found for this sprayer in the first crop stage and for the conventional crop-adapted PWM sprayer compared to the reference sprayer in all crop stages.

#### **7.08.P-We447 Environment Risk Assessment: How UAV Technology Could Contribute and Be Beneficial**

**Gaëlle Der Hagopian<sup>1</sup>**, **Rena Jutta Irene Isemer<sup>2</sup>**, **Sarah Hovinga<sup>3</sup>**, **Walter Mayer<sup>4</sup>**, **Martyn Griffiths<sup>5</sup>**, **Matthias Tempel<sup>4</sup>**, **Josef Exler<sup>4</sup>** and **Robin Sur<sup>6</sup>**, (1)EMEA Regulatory Science, Bayer AG - Crop Science Division, France, (2)Crop Science, Bayer AG - Crop Science Division, Germany, (3)Bayer AG - Crop Science Division, (4)Bayer AG - Crop Science Division, Germany, (5)Bayer AG - Crop Science Division, France, (6)Environmental Safety, Bayer AG - Crop Science Division, Germany

In the European Union, the sustainable use of pesticides is a major component of the agricultural framework. The future Sustainable Use Regulation considers precision farming and precision application as enablers of sustainable agriculture, helping to apply the right amount of the right product, at the right time, in the right place. Accordingly, precision application is an important approach helping to meet the goals of the European Commission's Green deal to reduce the risk and impact of pesticide use.

Which farming device(s) could then contribute here? Emerging technologies such as satellite imagery, data analytics, sensors, drones/UAVs (unmanned aerial vehicles), robotics, and artificial intelligence are key players in this context. For weed management (post-emergence application of herbicides) remotely gathered data by UAVs can be used by ground sprayer being equipped with a dedicated patch spraying setup or can be paired with a targeted UAV application. Both could be an opportunity for volume reduction of pesticides, by replacing a broadcast application with a precision application. With that, this helps to reduce environmental exposure (less drift and groundwater entry). Furthermore, this targeted application by UAV allowance provided, could also bring additional value, such as reducing soil compaction, fuel consumption, as well as the volume of water used.

The data resulting from precision application by UAV could then contribute to feed a realistic environmental risk assessment, using field-specific conditions. However, clarifications would be necessary on how this will concretely contribute to be part of the modelling and standardization of the environmental risk assessment. Data availability will be an important parameter to consider, particularly data ownership.

The scientific community is interested in having appropriate data available on drone technology, more precisely on broadcast application, as it represents the worst-case scenario regarding environmental impacts. To dive deeper into this, initiatives have begun around the world with industry participation (OECD, CLI, AEPLA), to obtain, among others, environmental data (drift data). We will present to which extent the UAV technology can provide precise data and share our proposal on how to best consider those data in the environmental risk assessment.

## **Track 8: Special Sessions**

### **8.01 Establishment of a Science-Policy Panel to Contribute Further to the Sound Management of Chemicals and Waste and Prevent Pollution**

#### **8.01.T-01 Background and Next Steps for the Open Ended Working Group and Establishment of the Intergovernmental Science-Policy Panel**

**Kevin Helps**, *UNEP Principal Scientific Officer, and Secretariat Science Policy Panel for the Continued Sound Management of Chemicals, Waste and Pollution Prevention, Kenya*

Background, steps already taken and what's next for the OEWG, and the process towards creating an Intergovernmental Science-Policy Panel for the Continued Sound Management of Chemicals, Waste and Pollution Prevention.

#### **8.01.T-02 Lessons Learnt From the IPCC and IPBES That Will Inform the Design and Operation of the New Science-Policy Panel - What Can We Expect to See Moving Forward**

**Robert Watson**, *Co-Chair Geo 7 and Chair of Technical Advisory Group to UNEP, United Kingdom*

Lessons learnt from the IPCC and IPBES that will inform the design and operation of the new science-policy panel, and what we can expect to see moving forward.

#### **8.01.T-03 What Questions Should Be Posed to the Global Academic Community, How Does the Community Go About Answering These Questions, and Is Consensus Important?**

**Hanna Andrea Rother**, *University of Cape Town, South Africa*

What questions should be posed to the global academic community, how does the community go about answering these questions, and is consensus important?

#### **8.01.T-04 Reflections on the Process of the Open Ended Working Group - Recommendations for Governments**

*Jason Weeks, IEH Consulting, United Kingdom*

As a science expert to the UK delegation for the 1.2 OEWG, what are your reflections on the process so far, and what recommendations do you anticipate for governments?

#### **8.01.T-05 How can the LCA Community Contribute to the Process?**

*Carla Caldeira, EC JRC, Italy*

How can the LCA Community Contribute to the Process?

#### **8.01.T-06 What Role Can Scientists Working in the Business Sector Play in This Process, and Why Is This Important?**

*Stewart Owen, Astrazeneca UK, United Kingdom*

What Role Can Scientists Working in the Business Sector Play in This Process, and Why Is This Important?

#### **8.01.T-07 The Importance of Including Early Career Scientists in the Process, and What They Can Contribute**

*Joanke van Dijk, Utrecht University, Copernicus Institute of Sustainable Development, Netherlands*

The importance of including early career scientists in the process, and what they can contribute.

#### **8.01.T-08 The Role That Professional Societies Can Play in the Process, and the Challenges and Benefits of This Type of Engagement**

*Camilla Alexander-White, Royal Society of Chemistry, United Kingdom*

The role that professional societies can play in the process, and the challenges and benefits of this type of engagement.

### **8.02.A Generating Experimental Data to Inform Effect Modelling: Challenges, Opportunities and Lessons Learned**

#### **8.02.A.T-01 Variability in Experimental Data: Mortality and Sublethal Endpoints**

*Anja Coors, ECT Oekotoxikologie, Germany*

The calibration and validation of effect models requires experimental data of high quality, i.e., data that are reproducible, among other criteria. Yet, natural biological and technical variability set limits to exact reproducibility of toxicity estimate values and to the prediction of biological parameters such as survival and reproduction. The design of calibration and validation experiments that support effect models should take into account the natural background variation in order to not generate datasets that only seemingly indicate low uncertainty or, in the contrary, appear to invalidate well-calibrated models. Another aspect in this context is that background variation, and thereby inherent uncertainty, varies among species and investigated endpoints, which renders it difficult to establish one default standard design for model calibration and/or validation. This talk will present examples for background variation and discuss underlying reasons and consequences in order to support a discussion of what can and what cannot be expected from experimental data when used in effect modelling.

#### **8.02.A.T-02 Perceptions of Goodness of GUTS Fits – Virtual Ringtest**

*Barbara Bauer<sup>1</sup>, Alexander Singer<sup>2</sup>, Oliver Jakoby<sup>2</sup>, Torben Wittwer<sup>2</sup>, Thomas G Preuss<sup>3</sup>, Johannes Witt<sup>3</sup> and Andre Gergs<sup>3</sup>, (1)Rifcon, Germany, (2)RIFCON, Germany, (3)Bayer AG - Crop Science Division, Germany*

Before a toxicokinetic-toxicodynamic model can be used for risk assessment, the quality of the model fit to the calibration data and its predictive performance on independent validation data should be evaluated. The evaluation should be based on both qualitative criteria (visual assessment) and quantitative Goodness-of-Fit (GoF) metrics. Cut-off values for some, but not for all, metrics are suggested by authorities to decide if model performance is acceptable according to a specific metric.

In this study we compare model evaluations based on either qualitative (visual inspection) or quantitative (GoF) criteria for General Unified Threshold models for Survival (GUTS) models. GUTS can be used to mechanistically model lethal effects of toxicants on non-target aquatic organisms in environmental risk assessment. The aims of this study are to identify whether quantitative criteria coincide with human perception of model performance, and if so, whether cut-off points for GOF metrics can be defined that meaningfully separate suitable from unsuitable models.

For this purpose, we calibrated and validated GUTS models on a large set of survival data from toxicity test. We created an online anonymous survey where >70 calibration and validation model fits were ranked by several dozen participants. Participants were asked to score model fits on a scale of 1 to 6 (i.e. good to poor model performance). The expertise of participants ranged from 'little experience in modelling' to 'modelling experts'. We compared expert evaluations from the survey to quantitative GoF metrics (e.g. NSME, PPC, SPPE and pseudo  $r^2$ ) and to an average GoF index that comprises information from all the metrics. Visual assessments and the average GoF index generally agreed on model performance, especially for very good and very poor fits. Interpersonal variation was largest among evaluators for intermediate quality fits. We found no systematic effect of affiliation or modelling experience on the visual evaluation score. All metrics showed some correspondence to visual scores. Only one of the metrics had a threshold-like relationship to scores, while others showed linear or exponential relationships. With these findings our study can contribute to the standardisation of methods in evaluating GUTS model performance and increase the understanding of GoF metric values in the GUTS context.

#### 8.02.A.T-03 Ring-Testing Experiments vs. Ring-testing Models: Results from CLE Algae Project

**Cecilie Rendal<sup>1</sup>, Johannes Witt<sup>2</sup>, Thomas G Preuss<sup>2</sup>, Ian Sims<sup>1</sup>, Roger Baetscher<sup>3</sup>, Sibylle Eck<sup>2</sup>, Monika Ratte<sup>4</sup>, Emily Scorgie<sup>5</sup>, Eric Bruns<sup>6</sup> and Roman Ashauer<sup>3</sup>, (1)Syngenta, United Kingdom, (2)Bayer AG - Crop Science Division, Germany, (3)Syngenta Crop Protection, Switzerland, (4)ToxRat Solutions, Germany, (5)Syngenta, Netherlands, (6)Environmental Safety, Bayer AG - Crop Science Division, Germany**

Ring testing of experimental methods for the generation of validation data for TKTD modelling is a significant undertaking that requires collaboration across industry, academia and regulators. The experimental workload is considerable as many laboratories execute complicated experiments in the highly co-ordinated and standardized structure required for a systematic ring test. The complexity of the experiments means that the subsequent evaluation of the data is equally complex. With the ongoing Crop Life Europe ring test for green micro-algae as an example, we here discuss the multifaceted considerations required in answering the simple question: *Does the experimental method generate data that is robust enough for model validation?* To fully answer this question, we highlight the correlation between our confidence in modelling results and our understanding of the characteristics of the underlying experimental data. We explore alternative metrics for quantifying uncertainty in the ring test data, and how each of these metrics relate to modelling results in a risk assessment perspective. Thus, we not only establish how our data relates to EFSA requirements for model validation, but we also explore how the variance in experimental data influences toxicokinetic parameters, and in turn how error in toxicokinetic parameters cascades through the modelling framework. With this exercise we attempt to understand and quantify both model uncertainty and experimental uncertainty within our modelling framework. This will not only help to define validity criteria for future generation of data using the experimental methodology, but also increase our confidence in the modelling approach and facilitate decision making through full transparency of the uncertainty from various sources.

#### 8.02.A.T-04 Developing Experimental “Standards” When Effect Modelling is the Aim

**Anna Huang<sup>1</sup>, Annika Mangold-Doring<sup>2</sup>, Paul van den Brink<sup>2</sup> and Jan Baas<sup>2</sup>, (1)Wageningen Environmental Research (WUR), Netherlands, (2)Wageningen University & Research, Netherlands**

Toxicity experiments play a fundamental role in generating data for the risk assessment of toxic chemicals. Usually, these experiments follow standards with strict lab conditions developed by various organizations, such as the United States Environmental Protection Agency (EPA) or the Organization for Economic Co-operation and Development (OECD). In these experiments the usual endpoint is an LC<sub>50</sub> (lethal concentration 50%) or a NOEC (No Observed Effect Concentration) which are time dependent, and do not provide any mechanistic information. Therefore the extrapolation potential of the test to real-life circumstances with time-varying concentrations in a dynamic environment, is very limited at best.

To address this issue, ecotoxicological effect models, such as toxicokinetic and toxicodynamic models (TKTD), have become increasingly popular in understanding the mechanisms of chemical toxicity and thereby greatly increasing the extrapolation potential of a laboratory test. Although TKTD models offer advantages in capturing and predicting toxic effects, their applicability is limited by the lack of specific experimental requirements for model calibration and validation.

To bridge this knowledge gap, we have developed a set of specific and feasible criteria for effect model calibration experiments, based on previous recommendations from the EFSA PPR Panel (EFSA PPR Panel (Panel on Plant Protection Products and their Residues) et al. 2018) and our own research. These criteria involve considering the biotransformation of chemicals in the toxicokinetic test, using an exposure concentration range that can produce 0 to 100% toxic effects, selecting effect-dependent observation time points with a minimum of three, and transparently reporting data to reveal inter- and intra-species sensitivity. Standardizing these experiments would enable various organizations, including academic and regulatory agencies, to utilize effect models to improve chemical risk assessment.

#### 8.02.A.T-05 Including Experimental Conditions in Parameter Uncertainty Estimation

**Florian Schunck<sup>1</sup> and Matthias Liess<sup>2</sup>, (1)Helmholtz Centre for Environmental Research (UFZ), Germany, (2)Department of System Ecotoxicology, Helmholtz Centre for Environmental Research (UFZ), Germany**

Risk assessment increasingly recognizes the need for New Approach Methods (NAMs) to evaluate the effects of toxicants (Côté, Darling, & Brown, 2016). Among these, mechanistic models can be used to predict the effects of unknown compounds because they rely on bio-physical relationships rather than purely statistical relationships. This, however, requires that the parameters describing the model are unbiased and have low unexplained variance.

Parameter uncertainty is reduced by increasing the amount of information presented to the model. Traditionally, this is done by increasing the number of replicates or by pooling of experiments from different laboratories. However, even highly standardized protocols such as the *Daphnia Reproduction Test* (OECD, 2012) leave some room for variability between laboratories, raising the question of whether variables are truly i.i.d. Another source of uncertainty is the loss of information between models and experiments. In the worst case, biased estimates can arise if the model is not an accurate description of the data generating process.

This work presents a simulation framework to assess **bias** and **uncertainty** by explicitly modeling the experimental conditions. Experimental protocols can be provided to the simulator in a spreadsheet-like style. In addition, the observational process can be accurately described, including details of the measurement, which may also be subject to interlaboratory variation. Working in such a framework has three major advantages: 1) The data generating process is more accurately described, reducing the estimate's bias. 2) The same model can be fitted on many more experiments, since the experimental condition is *just* another input variable. 3) The most important metadata of the experiment is stored by default, which improves data quality and reproducibility.



A discretized simplified dynamic energy budget (DEBkiss) (Jager, 2018) model is fitted on a chronic exposure dataset of *Daphnia magna* with and without considering the experimental protocol. The results show that the parameter estimates for resource distribution () and egg-mass significantly depend on the experimental protocol.

## 8.02.B Generating Experimental Data to Inform Effect Modelling: Challenges, Opportunities and Lessons Learned

### 8.02.B.T-01 Why Do We Care About Uncertainties When Designing New Experiments and How Should We Account for Them?

**Sandrine Charles**, *University Claude Bernard Lyon 1, France*

Generating experimental data to inform effect modelling is not a new topic as since it almost goes back to the very beginning of ecotoxicology. What is new is on one hand the models used to characterize the effects of exposure to chemical substances, mechanistically and accounting for both the concentration- and the time-dependency; on the other hand, the enormous amount of information accumulated over time, which is now available, in theory, to plan new experiments. In its early days, ecotoxicology was equipped with experimental protocols dedicated to hypothesis tests, unfortunately erroneously employed to define observed thresholds of toxicity. Although regulations have since evolved, recommending the use of dose-response models, experimental protocols have changed very little, focusing on the number of replicates rather than the number of tested concentrations, while the number of time points is fixed according to lab constraints instead of model needs. Statistical optimal design theory, however, allows the setting of experimental conditions (dose levels, measurement times...) in a way which optimizes the number of required measurements and organisms to obtain the desired precision of the results at constant cost. For example, showed that reliable nominal concentration may be fixed based on prior knowledge and literature research instead of on preliminary experiments. It quickly becomes clear that if experimental protocols are still not optimized for the use of dose-response models, they are even less so for mechanistic effects models. Despite this, we now know that these standard protocols, far from being optimal, may be sufficient for are sufficient to fit toxicokinetic–toxicodynamic models such as the reduced GUTS models. Even if some people identified optimal experimental designs for a toxicokinetic–toxicodynamic model based on a Bayesian method, we are still far from revolutionizing the environmental risk assessment. In this talk, I will illustrate how taking uncertainties into account is a promising alternative in the design of new experiments, without changing habits, neither in the way experimental data are collected, nor in the way they are used to fit models. I will give two examples: one with a dose response model to assess the toxicity of an herbicide on plant growth; a second with a GUTS model to assess cadmium effect on survival over time of gammarids.

### 8.02.B.T-02 Integrating Heterogeneous Data from Tissue to Population Level to Model Data-poor Species

**Peter Vermeiren**, *Natural Sciences and Environmental Health, University of South-Eastern Norway, Norway*

The widespread occurrence and enormous diversity of environmental pollutants could affect many non-target wildlife species, far beyond the limited number of ecotoxicological model species that are traditionally investigated. For many wildlife species, however, there are practical and ethical limitations to ecotoxicological data collection both in lab and field, resulting in poor data coverage for these species. Further, given the current push to reduce vertebrate toxicity testing, additional data are unlikely to become available in the near future. This raises real questions for the quantification of potential threats posed by environmental pollution to wildlife.

The formulation and combination of several mechanistic models, supported by databases derived from systematic, quantitative literature meta-analyses, could offer a solution to develop quantitative effect models for relatively data-poor wildlife species. Such effect models build on our understanding of ecological, physiological, and toxicological mechanisms which can be generalized across species, as well as integrate and harmonize best available data regarding observed patterns of exposure, accumulation and effects scattered across the scientific literature.

In this presentation, I will show some approaches employed to collect data and knowledge from scattered sources, and how to homogenise and integrate them into mechanistically based models. Along the way I will highlight opportunities to improve such approaches in the future as well as obstacles remaining. I will outline how meta-analyses and models could work together to allow for simulations and scenario analyses that cross multiple levels of biological organisation. This outline will be supported with examples from ongoing work on reptile species, where ecotoxicological data are particularly poorly available.

### 8.02.B.T-03 Seeing Variability in Experimental Data: Perks and Disadvantages from a Regulatory Perspective

**Alberto Linguadoca and Alessio Ippolito**, *European Food Safety Authority, Italy*

Growing calls (Topping et al., 2020) for increased realism in regulatory environmental risk assessment (ERA) frameworks have partially triggered the development of progressively more articulated ERA schemes (e.g., EFSA, in prep). In the area of pesticides, the need to incorporate realism and complexity in ERA may have effectively triggered a paradigm-shift, whereby reductionist approaches will be slowly replaced by more holistic frameworks (EFSA, 2021). Such transition may constitute a tremendous opportunity to boost the confidence and case-specificity in decision making. However, it may also require dealing with increasingly rich, complex and variable datasets, which may contribute to exacerbating the challenges of both risk assessment and management. We may be some time away from such a transition being taken up by EU regulatory frameworks. However, the ongoing trend toward increased complexity in ERA has effectively sparked the need to better contextualise challenges and opportunities related to using variable data in the context of regulatory risk assessment.

ERA relies upon consecutive tiers across which the problem formulation is narrowed down to increasingly specific regulatory questions. This paradigm, whereby realism, complexity and case-specificity of the assessment increase in a stepwise manner, may set the basis to identifying challenges and perks of relying upon variable data.

In my presentation I will give a regulatory perspective on how environmental risk assessors may be interested in constraining or exploiting intrinsically variable data depending on the specific regulatory question under assessment across the tiered system.

### **8.03 The European Green Deal (Chemicals Strategy for Sustainability)**

#### **8.03.T-01 Key question 1: Science-based decision-making (I)**

*Sabine Elisabeth Apitz, Integrated Environmental Assessment and Management, United Kingdom*

How do we avoid misuse of the phrase ‘science-based’, what does it mean, is all data useful data, and what level of information is sufficient to make informed decisions?

#### **8.03.T-02 Key question 1: Science-based decision-making (II)**

*Anna Lennquist, ChemSec, Sweden*

How do we avoid misuse of the phrase ‘science-based’, what does it mean, is all data useful data, and what level of information is sufficient to make informed decisions?

#### **8.03.T-03 Key question 2: Multidisciplinary and transdisciplinary solutions (I)**

*Joel Tickner, University of Massachusetts, Lowell*

Multidisciplinary and transdisciplinary solutions are called for, but how do we (as SETAC members and stakeholders) facilitate and achieve truly effective collaborations between different disciplines involved in the design, production, and assessment of chemicals, on what topics, and to what end?

#### **8.03.T-04 Key question 2: Multidisciplinary and transdisciplinary solutions (II)**

*Leo Posthuma, National Institute for Public Health and the Environment (RIVM), Netherlands*

Multidisciplinary and transdisciplinary solutions are called for, but how do we (as SETAC members and stakeholders) facilitate and achieve truly effective collaborations between different disciplines involved in the design, production, and assessment of chemicals, on what topics, and to what end?

#### **8.03.T-05 Key question 3: Modernised chemicals risk and alternatives assessment processes appropriate for the CSS (I)**

*Maurice Whelan, European Commission Joint Research Centre, Italy*

Which elements should be included in a modernised chemicals risk and alternatives assessment processes appropriate for the CSS’s stated goals? How to prioritise the investment of efforts between high-throughput hazard screening using ‘simplified’ NAMs-based approaches, and the more holistic, spatially and temporally resolved analyses needed to assess ecological effects of chemical mixtures in the environment? What are the CSS’s key science-data gaps to both inform decisions on chemicals and shape future chemicals that are Safe and Sustainable by Design?

#### **8.03.T-06 Key question 3: Modernised Chemicals Risk and Alternatives Assessment Processes Appropriate for the CSS (II)**

*Kristin Schirmer, Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology, Switzerland*

Which elements should be included in a modernised chemicals risk and alternatives assessment processes appropriate for the CSS’s stated goals? How to prioritise the investment of efforts between high-throughput hazard screening using ‘simplified’ NAMs-based approaches, and the more holistic, spatially and temporally resolved analyses needed to assess ecological effects of chemical mixtures in the environment? What are the CSS’s key science-data gaps to both inform decisions on chemicals and shape future chemicals that are Safe and Sustainable by Design?

#### **8.03.T-07 Key Question 4: Science-data Gaps to Inform Decisions and Shape Future Chemicals that are Safe and Sustainable by Design (I)**

*Jan Robinson, International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.), Belgium*

What are the CSS’s key science-data gaps to both inform decisions on chemicals and shape future chemicals that are Safe and Sustainable by Design?

#### **8.03.T-08 Key Question 4: Science-data Gaps to Inform Decisions and Shape Future Chemicals That Are Safe and Sustainable by Design (II)**

*Hans Sanderson, Aarhus University, Denmark*

What are the CSS’s key science-data gaps to both inform decisions on chemicals and shape future chemicals that are Safe and Sustainable by Design?

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